HISTOLOGICAL AND NUTRITIONAL STUDIES OF INTERNAL BREAKDOWN IN MANGO (*Mangifera indica* L.) FRUIT

By

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To my wife, Marie-Nadège J. Raymond, for so much sacrifice and self-denial.

To my son, Luc Junior, and my daughter, Laure-Michaëlle Raymond

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By

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Histological and nutritional differences between mango fruit with and without internal breakdown were investigated. The effects of Ca treatments on fruit and leaf Ca concentrations and on the incidence of the internal breakdown disorder were also determined. Visual and histological observations of healthy and disordered fruit indicated that jelly seed, soft nose, and stem-end cavity are different disorders. Fruit with jelly seed exhibited premature softening and senescence of the interior portion of the fruit mesocarp around the endocarp, whereas fruit with soft nose exhibited premature softening and senescence at the distal end of the fruit. Fruit with stem-end cavity exhibited necrosis and deterioration of the tissues at the peduncular extension of the fruit, resulting in the formation of a cavity. Histological observations with a light

microscope revealed cell wall deterioration in disordered tissues and a loss of cellular content, whereas cells of healthy tissues remained intact.

There were no differences in fruit Ca concentrations between healthy and disordered fruit. Disordered fruit contained significantly higher N and P concentrations than healthy fruit when the symptoms of internal breakdown first appeared. Except for P, no differences in nutrient concentrations were found between ripe fruit with and without internal breakdown. Fruit of 'Irwin' were less susceptible to internal breakdown than fruit of 'Tommy Atkins' or 'Van Dyke'. A higher number of 'Van Dyke' fruit were affected by internal breakdown than 'Tommy Atkins' or 'Irwin' fruit. Throughout fruit ontogeny, there was an inverse relationship between leaf and fruit Ca concentrations in 'Irwin', 'Tommy Atkins', and 'Van Dyke' mango trees. Leaf Ca concentrations increased and fruit Ca concentrations declined as the fruit developed. No significant fluctuations occurred in leaf N, P, and K concentrations during fruit development, whereas leaf Mg concentrations decreased with time. In general, the youngest fruit of all three cultivars contained higher N, P, K, Ca, and Mg concentrations than ripe fruit. Four weeks after fruit set, 'Irwin' fruit contained significantly higher Ca concentrations than fruit of 'Tommy Atkins' or 'Van Dyke'. However, when the fruit were ripe, there were no differences in Ca concentrations among the cultivars.

Biweekly foliar sprays of $CaCl_2$ to 'Irwin', 'Tommy Atkins', and 'Van Dyke' mango trees at 0, 4.6, 9.2, of 18.4 g/L, beginning four weeks after fruit set until the fruit were ripe, did not significantly increase the fruit Ca concentration or reduce the incidence of internal breakdown in the fruit. Significant relationships were observed between weekly foliar sprays of Cab'Y or Packhard at 0, 3.6, 5.4, or 7.2 g/L Ca and the concentrations of N, K, Ca, Zn, Mn, Cu, and B, in 'Tommy Atkins' fruit, especially at four weeks after fruit set.

CHAPTER I

INTRODUCTION

Mango is one of the most ancient cultivated fruit crops (Singh, 1960) and is among the most consumed fruits in the world. Mango is currently cultivated in 111 countries, 88 of which are mango exporters (Galán-Sauco, 1993). Mango was ranked fifth in world fruit production with world production estimated at 18,450,000 MT in 1994 (Anon., 1994). India is the world's major producer of mango (10,000,000 MT) and Mexico, Brazil, and Haïti are the three major producers in the Americas with 1,130,000 MT, 400,000 MT, and 230,000 MT produced, respectively, in 1994 (Anon., 1994). International trade of mango is primarily centered on imports to the United States, Europe, and parts of Asia (Galán-Sauco, 1993).

Mango production in the mainland of the United States is primarily localized in south Florida. In the almost 130 years since mango was introduced to the area, south Florida has become a significant mango producer and is considered to be a secondary center of diversity for genetic material and technical information (Knight and Schnell, 1993). Prior to Hurricane Andrew in 1992, the mango-cultivated area in Florida was estimated at 1,167 hectares (Anon., 1992). Mango production in Florida was estimated to have increased to 10,000 MT in 1992, from 6,250 MT in 1980 (Anon., 1992). This represents a 37.5% increase in 13 years. Despite damage caused by Hurricane Andrew, mango production contributed $ 1.7 million to Florida economy in 1995 (Anon., 1996). The area of mango cultivation is not likely to increase in Florida due to increasing imports, increasing urban pressure, and passing of the North American Free Trade Agreement (NAFTA).

Mango is mostly sold as a fresh fruit, which is perishable. This constitutes a limiting factor in international trade, which generally requires long distance transport and prolonged storage. Early harvesting has been adopted as an alternative to allow mango fruit to reach distant markets in acceptable conditions. Early harvested fruit are of lower quality, and are sold for lower prices than those that ripen on the tree. In addition, in several mango producing regions including Florida, a serious physiological disorder of mango fruit commonly called 'internal breakdown' is particularly devastating when fruit are allowed to ripen on the tree. Internal breakdown is a disequilibrium in the maturation of the fruit, characterized by the premature, partial ripening and/or deterioration of the mesocarp in the area surrounding the seed and/or at the proximal or at the distal end of the fruit. Fruit affected by this disorder have no commercial value.

When the incidence of internal breakdown is severe, in highly susceptible varieties, up to 80% of an annual crop can be lost, particularly if fruit are allowed to ripen on the tree. Some mango cultivars such as 'Alphonso' (Gunjate et al., 1982; Katrodia, 1988), 'Keitt', 'Tommy Atkins', and 'Van Dyke' (Malo and Campbell, 1978) are severely affected by internal breakdown. The exact cause of the disorder has not been determined.

Internal breakdown of mango fruit is referred to in different parts of the world as 'jelly seed', 'soft-nose', 'stem-end cavity' (Florida), 'tip pulp', 'spongy tissue' or 'soft center' (India), and 'yeasty fruit rot' or 'insidious fruit rot' (Malaysia). It is uncertain if these are different disorders or different symptoms of the same disorder. There are no descriptions in the literature of anatomical changes in the fruit associated with each of these symptoms. Describing the anatomical differences between jelly seed, soft nose, and stem-end cavity may indicate if these are different disorders or symptoms of the same disorder.

Young (1957) reported that soft-nose was first observed in Florida, in Palm Beach County in 1950, but the disorder or a similar one had been previously reported in India on 'Safeda', 'Maladaha', 'Bambai', and 'Lucknow' mango fruit (Verma 1950). Internal breakdown has also been reported in several other countries, including the Canary Islands (Galán-Sauco, 1984), Venezuela (Malo and Campbell, 1978; Wainright and Burbage, 1989), Malaysia (Lim and Koo, 1985), Australia (Haury, 1986; Mead and Winston, 1991; Wainright and Burbage, 1989), South Africa (Mead and Winston, 1991; Oosthuyse 1993; Wainright and Burbage, 1989), Sénégal (Burdon et al., 1991; Wainright and Burbage, 1989), Brazil (Van Lelyveld and Smith, 1979), and Mexico (Lakshminarayana et al., 1985).

Internal breakdown is difficult to detect prior to harvest due to the lack of accurate external clues. Also, the causal agent remains to be clearly identified. However, research suggests that the problem is related to a nutritional imbalance. Calcium (Ca) deficiency and, to a certain extent, N excess, are considered to be the most probable causes (Schaffer, 1994; Young et al., 1962; Young and Koo, 1969; Young and Miner, 1961). Factors such as excessive rain (Katrodia, 1988) or genetic susceptibility (Galán-Sauco et al., 1984; Mead and Winston, 1991) are suspected to result in the manifestation of the disorder.

Evidence is still lacking to make any final conclusion about the cause of internal breakdown. Young and Miner (1961) first suggested the possibility of a link between leaf Ca levels and the soft-nose disorder in mango fruit. Subsequent research implicated fruit Ca levels as a contributing factor in the development of internal breakdown in mango fruit (Burdon et al., 1991; Gunjate et al., 1979). However, a number of studies have failed to establish a relationship between Ca concentration in the fruit and internal breakdown (Krishnamurthy, 1981, 1982; Wainright and Burbage, 1989). Most previous studies on the relationship between nutrition and internal breakdown focused on ripe or at least fully-developed fruit. The available literature does not contain any information focusing on the

relationship between the concentration of mineral elements throughout fruit ontogeny and internal breakdown. If internal breakdown in mango fruit is caused by Ca deficiency, the deficiency probably occurs at a specific moment during fruit ontogeny. Thus, adequate timing and dosage of Ca supplied to the plants may prevent or minimize the occurrence of the disorder.

The main objectives of this study were to 1) investigate the alteration in the structure of the mesocarp cells in mango fruit exhibiting different symptoms of internal breakdown; 2) determine the relationship between leaf and fruit Ca concentrations and the occurrence of internal breakdown and; 3) test the effects of Ca treatments on the occurrence of internal breakdown. The ultimate goal of this study was to elucidate the cause of internal breakdown in mango, thus leading to a method for controlling the disorder.

This research was divided into three parts. The first part consisted of laboratory work evaluating the histological differences between healthy and disordered tissues, and to establish the possible differences among soft-nose, stem-end cavity, and jelly seed. The first part also included a histochemical test for Ca, to illustrate the possible differences in Ca concentrations between healthy and disordered tissues. The second part was a field study to observe mineral element concentrations in fruit and leaves throughout fruit ontogeny, and to test the effects of foliar applications of four concentrations of $CaCl_2$ on leaf and fruit mineral element concentrations, and on the incidence of internal breakdown in three Florida mango cultivars. The third part consisted of another field study to test the effects of two other Ca compounds, Cab'Y and Packhard, on 'Tommy Atkins' mango trees. Cab'Y is manufactured by Stoller Chemical Company and has 10% Ca as Ca-sucrose. Packhard is manufactured by Micro-Flo Company and contains 8% Ca as $CaCl_2$. The effects of the compounds on leaf and fruit mineral element concentrations, and on incidence of internal breakdown were investigated.

CHAPTER II

LITERATURE REVIEW

Habitat of Mango

Mango (*Mangifera indica* L.) is a member of the Anacardiaceae. The genus *Mangifera*, to which mango belongs, comprises about 69 species (Kostermans and Bompard, 1993), most of which are native to Southeast Asia (de Laroussilhe, 1980; Singh, 1960). *Mangifera indica* is the most commercially important and widely distributed species in the genus (Singh, 1960). The native range of mango is between 20° north and south of the equator in areas characterized by seasonally dry and wet climates. However, mango can be successfully grown outside its natural range (e.g. south Florida) provided that temperatures are not too limiting. In some cases, it is found north of the Tropic of Cancer, e.g. Canary Islands, Florida, and Israel. Mango is currently cultivated worldwide in most of the intertropical zone, except for areas with limiting climatic conditions. The optimum day temperature for mango development is between 23° and 27°C (de Laroussilhe, 1980; Schaffer et al., 1994). However, mango can withstand air temperatures higher than 40°C (de Laroussilhe, 1980), up to 48°C (Schaffer et al., 1994), and survive freezes of short duration depending upon the age and vigor of individual trees. Popenoe (1974) indicated that trees less than 5 years of age were killed by a temperature of -3°C. In the subtropics, low temperatures of 10-15°C stimulate flowering in mango trees (Schaffer et al., 1994; Whiley, 1993). Nuñez-Elisea and Davenport (1991) demonstrated that flowering occurred in containerized 'Tommy Atkins' mango trees subjected to 18°C during the day and 10°C at night for a minimum of 30 days with a 13-hour photoperiod.

Mango trees develop well in areas with varying amounts of annual rainfall. For mango growth and development, the distribution of the precipitation throughout the year is more important than the total amount of rainfall. Successful commercial mango production requires irrigation in areas where the average annual rainfall is less than 750 mm (de Laroussilhe, 1980). Mango possesses good adaptability to periodically flooded soil conditions. Larson et al. (1993) studied the effects of flooding on potted 'Tommy Atkins' and 'Peach' mango trees. A 14-day flooding period resulted in reduced CO_2 assimilation and root growth, and increased the number of hypertrophied lenticels. In that study, flood-induced lenticel hypertrophy was positively correlated with mango tree survival rates.

Nutritional Requirements of Mango

Mangos are adaptable to various soil types and characteristics such as pH, fertility, and depth. The species grows best in deep, loamy soils with good aeration and drainage. Consequently, nutritional recommendations vary among geographical regions and, sometimes, from site to site within the same region. Hence, it is difficult to generalize nutritional requirements for mango. For example, de Laroussilhe (1980) indicated that ideal concentrations of nutrients in the leaves of healthy mango trees are 1.35-2.32% N, 0.14-0.17% P, 0.64% K, 0.91% Ca, 0.26% Mg, 200 ppm Zn, Cu, Fe, 100 ppm Mn, and 75 ppm B. However, in Florida, Young and Koo (1969) considered 1.2-1.55% N, 0.08-0.175% P, 0.3-0.8% K, 2-3.5% Ca, and 0.15-0.40% Mg to be ideal foliar concentrations for mango. Similar recommendations have been made for mango orchards in Australia (Cull, 1991). Highly calcareous soils such as those in Florida and part of Israel are not ideal for mango production due to deficiencies of zinc (Zn), iron (Fe), manganese (Mn), and potassium (K) (Gazit 1969).

Nitrogen fertilizers may influence several developmental aspects in mango. Effects of N may vary according to the type of fertilizer used. For example, the NH_4 form promotes flowering (de Laroussilhe, 1980), while urea at 2-4% delays flowering if applied before flower differentiation (Haury, 1986). Urea has been reported to reduce flower panicle malformation and increase the number of hermaphroditic flowers and yield (Haury, 1986). Experiments conducted in Florida showed that N fertilization contributes to significant yield increases in mango (Young and Miner, 1960; Young et al., 1962).

Several researchers (Schaffer et al., 1994; Wainright and Burbage, 1989; Young and Miner, 1960, 1961; Young et al., 1962; Young and Koo, 1969) have pointed out that N excess can favor the incidence of soft-nose in mango. In Florida, it was observed that 80-90% of 'Kent' fruit were affected by soft-nose in trees that received 2.27 extra kg of N per year, while only 10% of the fruit in control trees that received approximately 0.36 kg of N showed the disorder (Young and Miner, 1960; Young et al., 1962). The incidence of the disorder was higher in sandy and acidic soils than in limestone soils with a high pH.

Information on mango responses to P have mainly focused on P deficiency. Root growth is among the processes most readily affected by P deficiency (de Laroussilhe, 1980). Leaves of P deficient plants become thick and hard, and their abaxial surfaces develop a reddish color (de Laroussilhe, 1980; Singh, 1960). Reports on the relationship between P deficiency and internal breakdown in mango are conflicting. Krishnamurthy (1981) observed a higher P content in the pulp of disordered fruit; however, Burdon et al. (1991) found no relationship between the disorder and the amount of P in the fruit tissue.

Mango requirements for K have not been clearly defined. According to Young et al. (1962), increased K fertilization resulted in no measurable yield increase and no decline in the incidence of soft-nose disorder in 'Kent' mango fruit. Subsequent

attempts to relate the incidence of internal breakdown to the K content of mature mango fruit yielded conflicting results. For example, Young et al. (1962) and Malo and Campbell (1978) found that levels of K in disordered, mature fruits were not correlated with the incidence of internal breakdown. However, Krishnamurthy (1981) found lower K levels in the pulp of affected mature fruits compared to healthy mature fruit, and Burdon et al. (1991) reported higher K concentrations in the mesocarp of disordered mangoes compared to that of non-affected mature fruit.

The optimum concentration of B in mango is poorly documented. De Laroussilhe (1980) indicated that ideal concentrations range of B in mango leaves is from 50-100 ppm. However, Young and Koo (1971) observed a 17-34 ppm range of B in Florida mango leaves. Boron deficiency in mango causes apical leaf necrosis, shortened internodes, swollen axillary buds, and premature leaf drop (Crane and Thomas, 1997; de Laroussilhe, 1980). In the fruit, the deficiency may translate into abnormal seed shape and darkened cotyledons (Crane and Thomas, 1997). Crane and Thomas (1997) indicated that B deficiencies can be corrected or prevented by making one annual foliar spray of 113 g of borax per tree. Boron deficiency has been linked to a mango fruit disorder called 'black tip' (Embleton and Jones, 1966). De Laroussilhe (1980) pointed out that one annual foliar application of borax was effective in controlling black tip. However, according to Srivastava (1963), the black tip disorder of mango is caused by toxic levels of ethylene, sulfur dioxide, and carbon monoxide from brick kiln fumes.

Anatomy, Morphology, and Ripening of Mango Fruit

Fruit anatomy and morphology. The mango fruit is a drupe which is laterally compressed to variable extents depending upon the cultivar (de Laroussilhe, 1980). Sizes vary from 2.5-30 cm long as do shape, taste, flavor, and color (Purseglove,

1968). Shapes from subglobose to elongate can be easily found (Singh, 1960). Among these extremes, other variants can also be observed and elliptical or reniform fruit are common. The apex is usually characterized by a small lateral projection called the beak. The beak may be prominent in some varieties or reduced to a small dot in others (de Laroussilhe, 1980; Singh, 1960). Some varieties produce fruits that are less than 100 g, other varieties can produce fruits of 1 kg or more. Mango varieties are classified into two categories: monoembryonic, with only one sexual embryo in their seed, which originated in India (Crane and Campbell, 1991), and polyembryonic, whose seeds have one sexual embryo and two or more nucellar embryos (de Laroussilhe, 1980; Samson, 1986; Schaffer et al., 1994; Singh, 1960), which originated in Indo-China (Crane and Campbell, 1991). There is a general agreement that cultivars with an Indian pedigree produce fruit with a highly colored skin, while those with an Indo-Chinese pedigree produce green to yellow colored fruit (Schaffer et al., 1994).

The mango fruit is a typical drupe consisting of the exocarp, the mesocarp, and the endocarp. The exocarp or skin is relatively thin in improved cultivars and may be less than 1 mm thick. It is green in the early stages of development and changes to yellow, yellow-green, orange, pink, reddish-purple, or purple at maturity (Singh, 1960). This color change may affect part or the entire surface of the fruit. The exocarp is composed of the outer epidermis and several layers of a thick-walled collenchymatous and small-celled epidermis (Roth, 1977). The exocarp also contains stomata and lenticels, and may become cutinized during fruit development (Roth, 1977). The density of lenticels is highest at beak of the mango fruit and lowest at the shoulders. The total number and the density of lenticels on the fruit exocarp appear to be cultivar-dependent in mango. A study of five mango cultivars in India revealed that the average number of lenticels varied from 19 to 35/cm^2 (Dietz et al., 1988). The cuticular thickness increased with time and varied among cultivars, the stomata remained functional up to 36 days after fruit formation.. The ranges of cuticular

thickness were 3.83 to 8.75 μm at the initial stage of fruit ontogeny, and 15.83 to 22.42 μm at the final stage of fruit development (Dietz et al., 1988).

The mesocarp or pulp (also called flesh) is the edible portion of the fruit. It is pale yellowish to orange in ripe fruit and may be variably fibrous and acidic. In ripe fruit of most commercial cultivars, the mesocarp is almost fiberless, sweet-acid to sweet, and very juicy (Popenoe, 1974). Fruit of these cultivars also are characterized by a sweet odor. In the wild forms, however, the mesocarp may be acidic and fibrous, and a turpentine-like aroma and flavor can be noted in these varieties (Purseglove, 1968). The mesocarp is almost entirely formed by parenchyma cells interspersed with vascular bundles, resin ducts, and tanniferous cells (Roth, 1977). Peripheral cells of the parenchyma are usually of a smaller size and contain less starch than cells of the inner layers, i.e. closer to the endocarp (Roth, 1977).

The endocarp or stone is usually laterally compressed and is fibrous (de Laroussilhe, 1980). Depending upon the variety, the fibers may be of variable length and tensile strength. They elongate in various directions to form an intricate network that extends into the mesocarp (Roth, 1977). Within the stone, there is a single seed consisting of the cotyledons surrounded by a papery testa.

Mango fruit ripening process. Typical of fleshy fruits, ripening in mango involves changes in physiology, color, texture, flavor, and aroma (Spencer, 1965). Fleshy fruits display two respiratory patterns during ripening. In the first group, called climacteric fruits, the ripening process is characterized by biochemical changes, fluctuations in the respiratory rate, and a significant production of ethylene. (Bryant and Cuming, 1993; Watada et al., 1984). Examples of climacteric fruits are apple, tomato, banana, papaya, and mango. In the second group, called non-climacteric fruits, there is no significant change in the respiratory rate, and ethylene production is minimal (Bryant and Cuming, 1993). Examples of non-climacteric fruits are cherry, grape, orange, pineapple, and strawberry (Spencer, 1965). Climacteric respiration in

mango and other climacteric fruit has three phases: the pre-climacteric, the climacteric, and the post-climacteric (Chaplin, 1986). The respiratory rate is low during the pre-climacteric phase, increases to reach a maximum in the climacteric phase, and decreases in the post-climacteric period (Bryant and Cuming, 1993; Chaplin, 1986). According to Lizada (1993), mango fruit may show respiratory rates exceeding 175 mg CO_2/2kg/hour at 25°C.

Ripening in mango fruit involves several biochemical processes associated with the activity of particular enzymes. These enzymes induce a series of physico-chemical changes (softening of the fruit tissues, increased total soluble solids, and development of pigments and aroma) as well as biochemical changes such as respiratory climacteric and ethylene production (Gomez-Lim, 1997). Softening of the fruit results from the solubilization of pectins in the middle lamella (Gomez-Lim, 1993; Lazan et al., 1993; Lizada, 1991) and solubilization and depolymerization of polyuronides in the cell wall matrix (Tucker and Seymour, 1991). In fruit affected by internal breakdown, the softening process is asynchronous (Lazan et al., 1993), i.e. part of the fruit softens more rapidly than the rest. In addition, the activity of cellulases, the enzymes assumed to be responsible for breaking down glucan molecules, increases significantly during ripening (Gomez-Lim, 1997). Cellulase action on the cell wall causes a loss of hemicelluloses from the cell wall matrix, and α-amylases, which catalyze the hydrolysis of starch molecules, become four times more active during ripening (Gomez-Lim, 1997). Sucrose phosphate synthase, the enzyme that catalyzes the synthesis of sucrose from hexose phosphate, increases in activity (Gomez-Lim, 1997). The activities of these enzymes are presumed to be responsible for the increase in sugar content that occurs during the ripening of the fruit.

Unlike other climacteric fruits, mango fruit produce little ethylene during ripening (Chaplin, 1986; Gomez-Lim, 1997). Lizada (1991) observed that internal ethylene levels in mango fruit are about 0.01 ppm before the climacteric phase, and

0.06 ppm during the climacteric period. However, Cua and Lizada (1990) had recorded ethylene levels of up to 0.35 ppm at 110 days after floral induction in 'Carabao' mangoes. This indicates that immature mangoes produce more ethylene than mature or ripe fruit. Two enzymes of the ethylene synthesis pathway become particularly active during the ripening process. The first enzyme, ACC synthase, catalyzes the conversion of S-adenosylmethionine (SAM) to 1-aminocyclopropane-1 carboxylic acid (ACC); the second, ethylene forming enzyme (EFE), or ACC oxidase, catalyzes the conversion of ACC to ethylene (Brady, 1992). Mango cultivars differ as to their pattern of ethylene production. For example, only one peak is observed in the ethylene production curve of 'Golek' fruit, while fruit of 'Carabao' exhibit two peaks, one during the climacteric period and the other at full ripeness (Lizada, 1991).

Ripening in mango may be influenced by several factors including stage of maturity at harvest, cultivars, exposure to ethylene or CO_2, and temperature. Medlicott et al. (1990) studied the responses of immature, half-mature, and mature 'Tommy Atkins', 'Ruby', and 'Amélie' mangos to 0, 0.1, 0.2, 0.4, 0.8, and 1.6 ml/L of acetylene, an ethylene analogue. Fruit were exposed to the treatments for 4, 8, 12, or 24 hours. Their results indicated a correlation between acetylene concentration and the exposure time necessary to initiate ripening in the fruit. For example, fruit treated with 0.8 ml/L acetylene ripened within 24 hours, while the 1.6 ml/L treatment required 8 hours. In that study, immature fruit failed to ripen completely despite increased softening and color development. Also, Seymour et al. (1990) conducted a study on the ripening of 'Amélie', 'Kent', and 'Sensation' mangoes harvested at three different stages of maturity and stored at 12°C for 21 days. They observed that ripening was more effectively delayed in immature fruit than in mature fruit. Also, cultivars responded differently to low temperature storage. 'Sensation' fruit ripened more rapidly irrespective of the stage of maturity at harvest. The authors attributed this observation to possible differences in the sensitivity to endogenous ethylene production among

cultivars. Bender et al. (1995) stored 'Tommy Atkins' mangoes at 12°C or 20° C for 21 days in 10%, 25%, and 45% CO_2 combined with 5% O_2. In that study, ethylene production rates were lower in fruit stored in 25% CO_2 than in the control fruit and those stored in 10% CO_2.

Calcium in Plants

Calcium is involved in several plant processes including root and shoot growth (Demarty et al., 1984; Hewitt and Smith, 1975), cell division (Hepler, 1986; Hepler and Wayne, 1985), fruit ripening and senescence (Brady, 1987; Eaks, 1985; Ferguson, 1984; Poovaiah et al., 1988; Spencer, 1965), enzyme activity (Delmer, 1987; Kauss, 1987), growth regulator responses (Eaks, 1985; Elliott, 1986), disease resistance and stress (Conway et al., 1994b; Palta, 1996; Yamazaki and Hoshina, 1995), signal transduction (Hepler and Wayne, 1985; Robinson et al., 1993; Wisniewski,1996), and physiological disorders (Bangerth, 1979; Shear, 1975). In the next sections, there is no attempt to make a complete survey of the existing literature. Rather, the aim is to present an overview of some specific aspects of the role of Ca in plant development.

Calcium uptake by plants. Calcium exists in the soil solution in its cationic form and is taken up by plant roots in the form of Ca^{2+} ions (Follett et al., 1981). There is a general agreement that Ca absorption by plant roots is passive and follows the influx of water (Kirkby and Pilbeam, 1984). Uptake is strongly influenced by the endodermis and is mostly restricted to the apical zone of the roots, and temporarily at the ramification site of roots (Bangerth, 1979). In these sites, deposits of suberin and lignin are reduced, and the Casparian strip is still underdeveloped. In older regions of the root, endodermal cells undergo additional deposition of suberin lamellae and lignification, which imposes a resistance to Ca entry into the endodermal cells (Esau, 1977). There, Ca moves across the endodermis from the cortex to the stele and the

xylem. Calcium entering the xylem may remain as a free ion, be complexed by organic acids, or bind to exchange sites on the xylem vessels (Hepler and Wayne, 1985).

Free Ca^{2+} ions in the xylem sap exchange with bound Ca from the xylem walls. The ascent of an individual Ca^{2+} ion from the source to the sink is not a continuous movement. It occurs by a series of adsorption and release between exchange sites (Clarkson, 1984). The distance that a Ca ion moves on average after release from one binding site before attaching to another is determined by the concentration of Ca^{2+} ions in the sap and the velocity of water movement in the xylem vessels (Hepler and Wayne, 1985). Although it is generally accepted that Ca moves with the transpiration stream, Gerard and Hipp (1968) correlated increased movement of Ca with low transpiration rates. This may have been the result of root pressure, which can also play a role in Ca movement, especially at night when transpiration stops. The importance of root pressure in Ca transport was established by Palzkill and Tibbitts (1977) who showed that the inner leaves of cabbage heads with a low transpiration rate were unable to obtain adequate amounts of Ca without root pressure. These authors further pointed out that high transpiration rates favored Ca transport to the outer leaves at the expense of the inner leaves.

Xylem ramifies and develops in leaves and other organs along with the growth of the stem (Esau, 1977). In growing organs, new Ca binding sites form continuously as a result of cell division and cell elongation (Clarkson, 1984). These new sites represent new sinks for Ca. Consequently, the demand for Ca in young organs, e.g. young mango fruit, is normally high, as new binding sites form due to cell division and elongation. In fruits of most species, xylem is mostly rudimentary (Esau, 1977) and nutrient transport occurs mainly in the metaxylem vessels. As a fruit grows, the ability of the xylary elements to provide nutrients to the organs decreases. As a consequence, fruit become more and more dependent on the phloem for nutrient uptake as they develop (Clarkson, 1984). Calcium appears to be immobile in the phloem. Because of

the low mobility of Ca in the phloem, the internal distribution of nutrients is impaired, and developing fruit may suffer from Ca deficiency if they are not continuously supplied with Ca. In the absence of a functional xylem, Ca movement is achieved by migration along the xylem walls or possibly by root pressure delivery of xylem sap.

Calcium in cell division. The role of Ca in cell division was first indicated at the beginning of the twentieth century (Jones and Lunt, 1967). Jones and Lunt (1967) reported that Ca is required for normal mitosis. This is in agreement with Hepler's (1986) observation of the inability of cells to form new cell plates during cell division without Ca. Research indicates that forming cell plates contain considerable amounts of Ca^{2+} ions. The discovery by Weisenberg (1972) of the depolymerization of microtubules by elevated levels of Ca^{2+} ions brought more support for the role of Ca in mitosis. Hepler and Wayne (1985) indicated that the membrane-associated Ca concentrations decrease a few minutes before cells enter anaphase.

Variations in intracellular Ca levels may promote or depress specific phases of cell division. Artificial manipulation of Ca availability during cell division with the chelator ethylenediaminetetraacetic acid (EDTA), for example, demonstrated that it is possible to stop or extend metaphase (Hepler and Wayne, 1985). At least 1 μM Ca^{2+} is required for anaphase to take place. Lower concentrations can extend metaphase.

Calcium in fruit ripening. Ripening of horticultural crops has been defined as the "composite of the changes that occur from the latter stages of growth and development through the early stages of senescence and that results in changes in composition, color, texture, or other sensory attributes" (Watada et al., 1984, p. 20). Ripening is associated with structural changes such as loosening of cell walls and diminution of cell cohesion. Other processes of ripening in climacteric tissues or organs are increased respiration rates and ethylene production (Bryant and Cuming, 1993; Spencer, 1965). Calcium displays functional control of several of these processes, in particular respiration and softening.

1. *Role of calcium in respiration.* The Ca^{2+} ion is associated with the exterior surface of the plasmalemma and plays a significant role in membrane stability and cell integrity (Kirkby and Pilbeam, 1984). By maintaining the integrity of cellular membranes, Ca indirectly affects the respiration rate in ripening tissues. Membrane stabilization results from links between Ca and the phosphate groups of phospholipids, and the carboxylate groups of proteins at the surfaces of the membranes (Clarkson, 1984). In the lipid bilayers, Ca binds to negatively charged phosphate groups of phospholipids and induces a tighter packing of these molecules. Ferguson (1984) indicated that increased packing of phospholipids in cellular membranes resulted in reduced membrane permeability. Thus, Ca in cell membranes is essential to maintain selectivity of ion transport. In the case of Ca absence or deficiency, membranes become leaky and solutes are lost from the cytoplasm (Hepler and Wayne, 1985; Poovaiah et al., 1993). Faust and Shear (1972) observed an inverse relationship between respiration of apples and flesh Ca concentration. An increase in respiration was observed below 110 mg kg^{-1} flesh Ca. In addition, Bramlage et al. (1974) reported declining post-climacteric respiration in apples with increasing Ca concentrations from 0.4 to 1.3 mg kg^{-1}.

Calcium can regulate respiration by controlling the flux of initiator molecules (phosphate) or of respiratory substrates (malate) across the cellular membrane. This Ca-related regulation of respiration has been evidenced by its effects on two enzymes involved in the respiratory process, particularly NADH dehydrogenase and NAD kinase, which are controlled by cytoplasmic Ca (Kauss, 1987; Lee and Leagood, 1993). NADH dehydrogenase is responsible for the oxidation of NADH to NAD, generating electrons and protons to complex I of the electron transport pathway (Lee and Leagood, 1993). This enzyme is activated by micromolar levels of Ca and is inhibited by Ca chelating agents such as ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) (Bryce and Hill, 1993). Calcium effects on

respiration may be due to its role in maintaining membrane integrity (Conway and Sams, 1987). Low levels of Ca, resulting in increased membrane permeability, can favor increased respiratory rates. Thus, low levels of Ca in fruit tissue can trigger the activity of NADH dehydrogenase, which can translate into a rise in respiration. This regulatory effect of Ca levels on respiration was also observed in 'Fuerte' avocado fruit (Tingwa and Young, 1974).

2. *Role of calcium in fruit softening*. Softening of fruit tissue is a result of the destabilization of the pectic matrix of the plant cell wall, mainly made of pectins and Ca pectate (Ferguson, 1984; Lee and Leagood, 1993). During the ripening process, softening is accompanied by losses in sugars from the cell wall, solubilization of pectins, and dissolution of the middle lamella (Brady, 1987; Ferguson, 1984). Thus, cell wall loosening and depressed cell cohesion are corollaries of softening.

The principal role of Ca in cell wall structure is the cross-linking of pectic polymers, mainly in the middle lamella (Demarty et al., 1984; Ferguson, 1984). Increased Ca concentrations in fruit tissue reduce the rate of fruit softening, and consequently of fruit ripening and senescence. Ferguson (1984) indicated that high concentrations of Ca lead to firmer fruit. Also, Conway and Sams (1987) observed that post-harvest applications of Ca to 'Delicious' apples by the pressure infiltration technique maintained fruit firmness. Similar observations were made by Eaks (1985) with 'Fuerte' and 'Hass' avocado fruit, to which they applied $CaCl_2$ by vacuum infiltration at 6.495 kPa for 1 minute. These results are consistent with observations made of mango (Tirmazi and Wills, 1981), sweet cherry (Facteau et al., 1987), apple (Glenn and Poovaiah, 1990), strawberry (Cheour et al., 1991), and papaya (Qiu et al., 1995).

Calcium Involvement in Plant Disorders

Calcium-related physiological disorders are common in horticultural crops. According to Shear (1975), about 24 different disorders observed in fruits and vegetables are caused by Ca deficiency. The best known of these disorders is 'bitter pit' of apples (Bramlage, 1994; Ferguson and Watkins; 1989 Maynard, 1979; Shear, 1975). Other Ca-related disorders include 'blossom-end rot' of tomato (Battey, 1990; Gerard and Hipp, 1968; Shear, 1975), 'cork spot' of pear (Curtis et al., 1990; Raese, 1989, 1994), 'end-spot' of avocado (Shear, 1975), 'blackheart' of celery (Shear 1975; Bangerth 1979; Battey 1990), and 'translucent flesh' of mangosteen fruit (Pankasemsuk et al., 1996). Several of these disorders can be either prevented or their occurrence minimized by Ca application.

Calcium can be supplied to plants in several ways. Application of limestone ($CaCO_3$), gypsum and other Ca fertilizers to soils are the oldest methods of supplying plants with Ca. Whole plants or plant organs can also be provided with Ca by several other methods either as pre-harvest or as post-harvest applications. These methods include foliar sprays (Krishnamurthy, 1982; Schaffer, 1994), dipping of plant organs into Ca-containing solutions (Gunjate et al., 1979), and infiltration by pressure or under vacuum (Conway and Sams, 1987; Conway et al., 1994a, 1994b; Tirmazi and Wills, 1981). Krishnamurthy (1982), in India, studied the effects of pre-harvest foliar sprays of 5000 ppm Ca and 500 ppm B alone or in combination, on the occurrence of spongy tissue in 'Alphonso' mangoes. The treatments did not affect the incidence of the disorder or the fruit Ca level. Dipping the fruit in 5000 ppm $CaCl_2$ solutions at different temperatures did not reduce spongy tissue nor increase fruit Ca level. Those results are in contrast with observations by Gunjate et al. (1979), who reported that dipping 'Alphonso' fruit in 0.5% or 2% $CaCl_2$ or $CaNO_3$ significantly reduced spongy tissue and increased the Ca concentration in the fruit.

Experiments with apples indicated that calcium can be successfully provided to fruit by infiltration techniques, which are considered more effective than dipping for controlling 'bitter pit' in apples (Conway et al., 1994a, 1994b). Tirmazi and Wills (1981), in Australia, conducted a survey of pressure $CaCl_2$ infiltration at 0%, 4%, 6%, and 8%, into 'Kensington Pride' mangoes. The treatments resulted in delayed ripening of the fruit and increased Ca content in both the peel and the mesocarp. Also, Conway and Sams (1987) subjected 'Delicious' apples to pressure infiltration (68.95 kPa) with $CaCl_2$, $MgCl_2$, or $SrCl_2$. The fruit were stored for 5 months at 0°C and then inoculated with *Penicillium expansum*. They observed that Ca was more effective than Mg or Sr in reducing fruit decay, suppressing ethylene production, and maintaining fruit firmness. In a subsequent experiment conducted during a 3-year period with 'Golden Delicious' and 'Delicious' apples subjected to post-harvest pressure infiltration with 0%, 4%, 6%, and 8% $CaCl_2$ for 6 minutes, Conway et al. (1994a) confirmed these observations. Calcium infiltration techniques may, however, present a few disadvantages such as skin injury which can increase the predisposition of fruit to microbial infection (Conway et al., 1994a; Tirmazi and Wills, 1981; Wills et al., 1988).

Internal Breakdown of Mango Fruit

The first report of a physiological disorder affecting the internal tissues of mango fruit dates back to 1950 when Verma reported losses of up to 25% of the total crop of four mango cultivars in India. The disorder, called 'tip pulp', mostly affected fruits nearing ripeness and was characterized by yellowing at the fruit tip. Fruit later turned grayish and the pulp softened. Attempts to isolate pathogenic organisms from the affected tissues were unsuccessful. As previously mentioned, internal breakdown is a term that encompasses several physiological disorders affecting mango fruit, such as

soft nose, flesh breakdown, jelly seed, stem-end cavity, spongy tissue or soft center, internal necrosis, yeasty fruit rot or insidious fruit rot.

Jelly seed is a breakdown of the mango fruit mesocarp in the area located around the endocarp or stone that is undetectable by external examination. Jelly seed-affected pulp appears to ripen at a faster rate than the rest of the fruit and is a darker yellow color. Advanced stages of the disorder are characterized by brown-colored and water-soaked tissues affecting a large portion of the mesocarp (Wainright and Burbage, 1989; Winston, 1984). *Soft nose* is a breakdown of the mesocarp at the fruit apex that causes a differential ripening of this portion of the fruit. As in jelly seed, the affected portion ripens faster than the rest of the mesocarp. When the disorder has progressed to advanced stages, it can be easily detected by touching the apex which is softer than the rest of the fruit (Burdon et al., 1991; Wainright and Burbage, 1989; Winston, 1984; Young, 1957). *Stem-end cavity* is a breakdown of the vascular connections between the fruit mesocarp and the peduncle resulting in the formation of a cavity at the proximal end of the fruit. The first indication of stem-end cavity in affected tissue is the appearance of browning in the proximal mesocarp. In advanced stages, a cavity is formed between the endocarp and the peduncle, and the affected tissue becomes dark brown or even grayish, particularly around the cavity. At this stage, the breakdown also affects the interior of the mesocarp, producing additional symptoms similar to those observed with jelly seed. In later stages, stem-end cavity can be detected at the proximal end of the fruit, which becomes when pressure is applied (Malo and Campbell, 1978; Mead and Winston, 1991; Wainright and Burbage, 1989; Winston, 1984).

Possible causes of internal breakdown in mango. The occurrence of soft-nose was first reported in Florida in 1957 (Young, 1957). Similar to observations by Verma (1950), Young found that the incidence was greatest in fruit close to ripeness and that the disorder was non-pathogenic. In that experiment, 7.7%, 9.6%, and 11.9% of

'Haden', 'Kent', and 'Zill' fruit were affected when N application rates were 90, 180, and 360 g/tree, respectively. Subsequent experiments (Young and Miner, 1960 and 1961; Young et al., 1962) also correlated increased incidence of soft-nose with high levels of N fertilization. The incidence of the disorder was higher in acidic soils with low Ca contents than in calcareous soils with a high pH and Ca content. These findings represented the first indication of a possible role of Ca deficiency in soft-nose.

In a 4-year experiment conducted in Florida, Malo and Campbell (1978) studied the effects of four levels of N and three levels of K applied to the soil on the incidence of internal breakdown in 'Tommy Atkins' mango fruit. Malo and Campbell (1978) could not correlate N or K treatments with the incidence of the disorder. They suggested a possible relationship between occurrence of the disorder and cultural practices, such as over-irrigation and excessive fertilization. In 1981, Katrodia and Rane made similar observations in a study of 'spongy tissue' of 'Alphonso' mango in India. They found that the disorder occurred with significantly higher intensity in vigorous trees than it did in weak trees. Lad et al. (1992), reported that N, P, and K treatments did not significantly influence the occurrence of spongy tissue in 'Alphonso' mango fruit in India.

The incidence of soft-nose has been correlated with high N and low Ca concentrations in the leaves, but no relationship was found between foliar levels of P, K, or Mg and the occurrence of the disorder (Young et al., 1962). In a study of the chemical composition of disordered 'Alphonso' mango fruit, Krishnamurthy (1981) found that there were no significant differences in Ca, Mg, and Na concentrations between affected and non-affected pulp of 'Alphonso' mango. That experiment also showed that disordered pulp had a higher concentrations of pyruvic acid, α-ketoglutaric acid, and acetaldehyde than healthy pulp. Malic enzyme and pectin methylestherase activities were also higher in the flesh of disordered fruit compared to that of healthy fruit.

In addition to Ca deficiency, there are indications that environmental factors may influence the incidence of physiological disorders in mango fruit. There is a general agreement that 'black tip' in mango is associated with the proximity of brick kilns, which produce fumes that are apparently toxic to mango trees (de Laroussilhe, 1980; Singh, 1960; Srivastava, 1963;). Moreover, Gunjate et al. (1982) observed an increased incidence of internal breakdown in 'Alphonso' mango exposed to sunlight, and Lim and Koo (1985) reported that, in Malaysia, the incidence of yeasty fruit rot in mango fruit was correlated with drought.

Orchard management practices, including irrigation and ground cover, have also been considered as potential causes of internal breakdown in mango fruit. However, very little research had been conducted on the effects of irrigation practices on internal breakdown. The few published research results are somewhat conflicting. Malo and Campbell (1978) concluded that the incidence of internal breakdown in 'Tommy Atkins' was not related to over-irrigation. Farre and Hermoso (1993) came to similar conclusions in a study of the cultivar 'Sensation' subjected to different irrigation treatments. However, Katrodia (1988), in India, reported that one or two rains per week-prior to harvest increased the susceptibility of 'Alphonso' fruit to spongy tissue. In that study, the relationship among available soil moisture, relative humidity, and the percentage of affected fruit was not reported.

Experiments that explored the effects of ground cover on the incidence of internal breakdown in mango are conflicting. For example, Katrodia (1988) reported that sod-culture resulted in an increased incidence of spongy tissue in tree-ripe 'Alphonso' fruit. In contrast, Lad et al. (1992) observed that the incidence of spongy tissue was significantly lower in 'Alphonso' fruit harvested from trees associated with sod-culture and grass mulch (34.5% and 21.62% respectively) than in control trees (50%). Both sources attributed their findings to an effect of the ground cover on reducing the temperature of the soil surface.

Genetic factors have been implicated in the development of internal breakdown and may be a major factor in developing control strategies for the disorder. Internal breakdown in mango fruit mostly occurs in Indian cultivars or those with an Indian pedigree (Schaffer, 1994). Galán-Sauco et al. (1984) and Mead and Winston (1991) also indicated that the disorder is cultivar-dependent. This view is evidenced by observations made in India by Ram et al. (1988), who surveyed the susceptibility of several mango cultivars to different disorders. They reported that the number of fruit affected by internal necrosis varied according to cultivar. The cultivar Langra did not exhibit symptoms of internal necrosis while 'Dashehari', 'Chausa', 'Safeda Lucknow', and 'Fajri' were prone to the disorder. However, all genotypes observed in that study were susceptible to black tip.

The mango cultivar Alphonso is well known for its susceptibility to spongy tissue. However, other cultivars such as 'Doodhapedha,' 'Goamankur', 'Vellaikolamban', 'Suvarnarekha', 'Fernandin', and 'Olour' also suffer from this disorder to varying extents (Katrodia, 1988; Lad et al., 1992). Cultivars such as 'Rajapuri' (Katrodia, 1988), 'Pairi', 'Kesar', 'Neelum' (Lad et al., 1992), 'Banganapalli', 'Kalapardy', and 'Janardham Pasand' (Iyer and Subramanyan, 1992) show no susceptibility to spongy tissue. This indicates that genetic factors are involved in the occurrence of physiological disorders in mango fruit. Breeding programs in India have already produced promising hybrids such as 'Hybrid 10' and 'Hybrid 13' (Iyer and Subramanyan, 1992) that are currently being evaluated for resistance to internal breakdown.

Control Measures for Internal Breakdown

Based on field and packinghouse observations, suggestions for reducing the incidence of internal breakdown in mango include early harvesting (Mead and Winston,

1991; Van Lelyveld and Smith, 1979; Winston, 1984), discarding non-bleeding fruits, i.e. those not exuding latex (Mead and Winston, 1991), or avoiding exposure of fruit to sunlight (Gunjate et al., 1979).

A small amount of research has focused on reducing internal breakdown with Ca applications. Young et al. (1962) reported 45%, 35%, and 27% reduction of soft-nose in 'Kent' fruit by soil application of $Ca(NO_3)_2$, $CaCO_3$, and gypsum, respectively. In a study with 'Alphonso' mango, Gunjate et al. (1982) observed that preharvest dipping of fruit in solutions containing $CaCl_2$, or $Ca(NO_3)_2$ significantly reduced the incidence of spongy tissue. In that study, $CaCl_2$ was more effective than $Ca(NO_3)_2$ and preharvest treatments gave better control than postharvest treatments. However foliar sprays of $CaCl_2$ (5,000 ppm) or H_3BO_4 (500 ppm) alone or in combination, at monthly intervals starting from fruit set until fruit maturity, did not significantly reduce the incidence of the breakdown in 'Alphonso' mango (Krishnamurthy, 1981).

CHAPTER III

HISTOLOGICAL OBSERVATIONS OF INTERNAL BREAKDOWN IN MANGO (*Mangifera indica* L.) FRUIT TO DISTINGUISH JELLY SEED, SOFT NOSE, AND STEM-END CAVITY

Introduction

Internal breakdown is the term generally used to refer to one or more physiological disorders in mango fruit characterized by uneven ripening of the fruit mesocarp, in which the interior of the mesocarp ripens faster than the exterior of the mesocarp. Internal breakdown (also known as flesh breakdown or internal flesh breakdown) is most often referred to as jelly seed, soft nose, or stem-end cavity (SEC). Although internal breakdown has been known to occur in mango for more than 50 years, the different symptoms have not been accurately described in the scientific literature. Soft nose has been considered to be similar to tip pulp (Young, 1957), or as an advanced stage of flesh breakdown (Malo and Campbell, 1978) or of SEC (Winston, 1984). In Indonesia, soft nose and tip pulp are considered to be identical to yeasty fruit rot or insidious fruit rot (Lim and Koo, 1985). Also, Galán-Sauco et al. (1984) described soft nose as a combination of jelly seed and SEC. In addition, the description of flesh breakdown symptoms by Malo and Campbell (1978) is very similar to SEC symptoms described by Mead and Winston (1991). Krishnamurthy (1981) and Subramanyam et al. (1971) used the term internal breakdown to describe the spongy tissue disorder or soft center in 'Alphonso' mango fruit.

Soft nose in mango fruit can be described as a partial ripening of the mesocarp at the distal end of the fruit. At the early stage of the soft nose disorder, a yellow area

is defined between the apex of the stone and the exocarp of the fruit. Soft nose primarily affects nearly fully developed fruit of certain cultivars, e.g. 'Tommy Atkins', and causes an extreme softening of the mesocarp, which becomes paste-like in the advanced stage of the disorder. At its early stage, the disorder can be diagnosed only by cutting open the fruit, but when it reaches the advanced stage, soft nose can be detected by pressing the fruit apex, which is usually softer than the other parts of the fruit (Fig. 3-1).

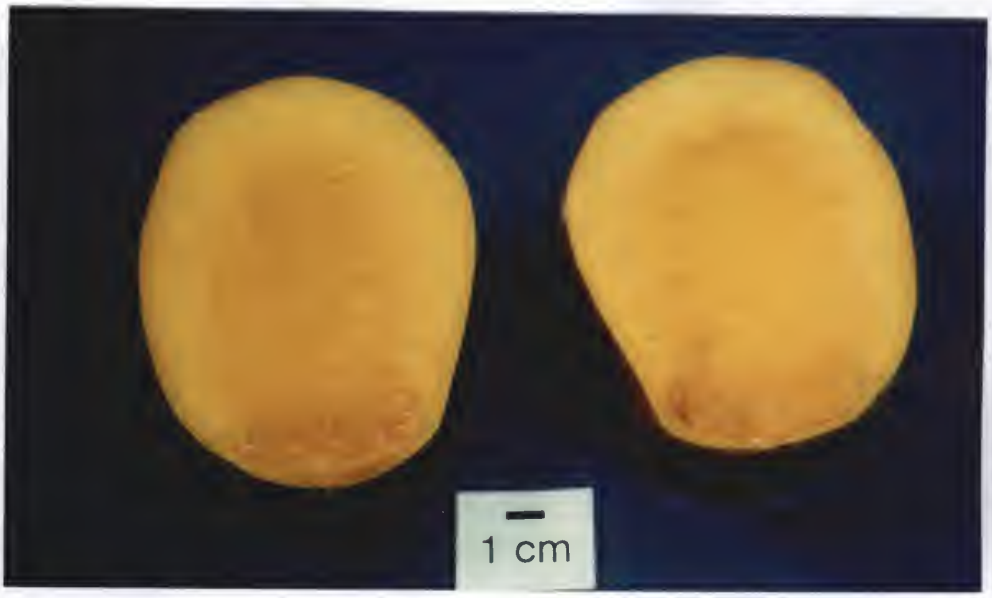
Jelly seed affects the interior of the mesocarp of the fruit, i.e. the portion of the mesocarp that is closer to the endocarp or stone. The jelly seed disorder is first characterized by a more pronounced yellow color of the affected area compared to the rest of the mesocarp, which remains whitish or pale green in young fruit. As the symptoms develop, the yellow color intensifies and reaches a larger portion of the mesocarp. The affected area eventually becomes brown and softens to the point of having the consistency of jelly (Fig. 3-2).

Stem-end cavity is a disorder characterized by the formation of a cavity in the proximal area of the fruit resulting from the deterioration of the vascular tissues between the proximal end of the stone and the fruit peduncle. Similar to soft nose, the only accurate method for detecting SEC is by cutting open the fruit. The affected tissues turn a brownish color at the earliest stage; then a small cavity forms as necrosis develops around the cavity. The interior of the mesocarp turns yellow or orange, whereas the exterior of the mesocarp remains whitish or pale yellow. The cavity later enlarges as the fruit reaches the advanced stages of the disorder (Fig. 3-3).

The scientific literature contains little information on the histopathology of internal breakdown. It has been reported that cell separation and cell wall degeneration occur in disordered mesocarp of fruit affected with soft nose, whereas cell cohesion is maintained in the healthy mesocarp (Burdon et al., 1991). It is not known if jelly seed, soft nose, and SEC are different disorders or, as suggested by Winston (1984),

Fig. 3-1. A mango fruit affected by the soft nose disorder.

Fig. 3-2. A mango fruit affected by the jelly seed disorder (advanced stage).


1 cm

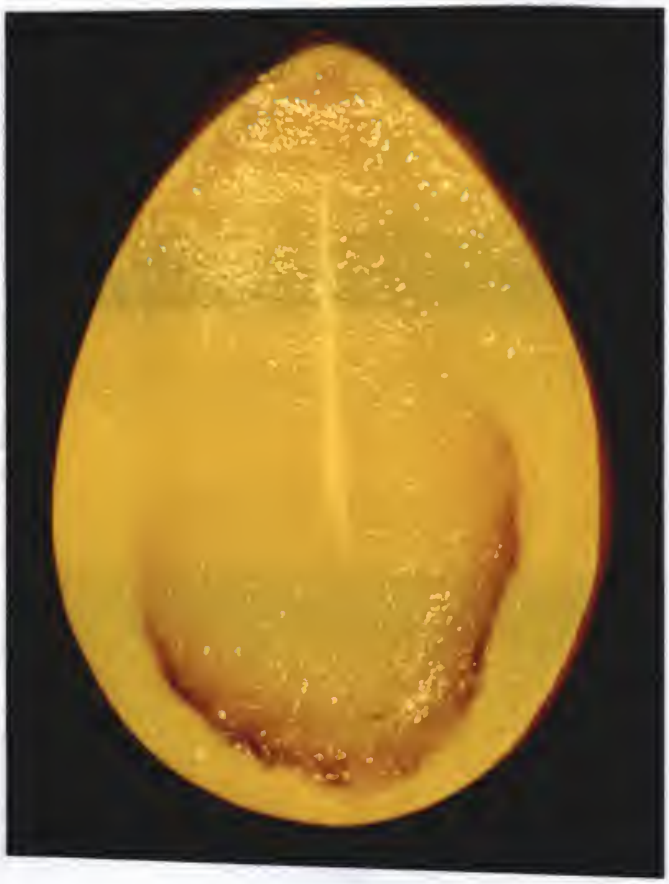


Fig. 3-3. A mango fruit affected by the stem-end cavity disorder.

different manifestations of the same disorder. Most descriptions reported in the literature are based on visual observations of mature and/or ripe fruit. One disadvantage with such procedures may be that, when the disordered fruit can be seen with the naked eye, symptoms are probably at advanced stages and, consequently, the possibility to distinguish them is greatly reduced. A study aimed at early detection of the symptoms of internal breakdown may allow jelly seed, soft nose, and SEC to be distinguished from each other to determine if they are different or the same disorder. The purpose of this study was to observe the disorders from their early stages of development, to attempt to discern histological differences or similarities among jelly seed, soft nose and SEC, and between disordered and healthy tissues.

Materials and Methods

Collection of samples. Fruit samples were collected from 'Tommy Atkins', 'Irwin', and 'Van Dyke' trees at 2-week intervals in 1995, and once a week from 'Tommy Atkins' trees in 1996, beginning 4 weeks after fruit set (WAFS) and continuing until the fruit were ripe. To assess the type of disorder, one transverse cut was made with a sharp knife at the proximal end of the fruit and two lateral cuts were made on each of the wider flat sides of each fruit. The transverse cut was made immediately under the skin, to expose the tissues in the peduncular extension and to assess the presence of SEC. In this study, the term peduncular extension is used as a synonym of proximal end to refer to the vascular connections located between the peduncle and the stone of the mango fruit, where SEC symptoms generally develop. Two other cuts were made on each flat side of the stone to expose the interior of the fruit so that jelly seed or soft nose could be detected. The symptoms were visually rated as early, intermediate, and advanced. For SEC, the rating of the symptoms was based on the presence and/or the size of the cavity at the proximal end of the fruit. Early

symptoms of SEC were those in which no visible cavity was formed at the peduncular extension of the fruit, but where the vascular tissues in that area had partly or completely turned a brownish color. Symptoms of SEC were considered as intermediate when a 0.1 to 0.5 cm diameter cavity was visible at the proximal end of the fruit, accompanied by a necrotic area around that cavity. Symptoms were ranked as advanced when the cavity was larger than 0.5 cm diameter and was accompanied by deterioration of the fruit mesocarp around the stone.

For jelly seed and soft nose, early symptoms were characterized by a pronounced yellow coloration of the mesocarp around the stone (jelly seed) or at the distal end or at the sinus of the fruit (soft nose), indicating that the senescence process had been initiated. Intermediate symptoms were those in which the disordered mesocarp had an orange color, whereas the surrounding tissue (exterior of the mesocarp) was still white or pale yellow, indicating that the exterior of the mesocarp was not ripe. Symptoms of jelly seed and soft nose were considered as advanced if the mesocarp showed advanced deterioration and/or discoloration. All fruit showing a combination of SEC and jelly seed symptoms were considered as SEC, and those with a combination of jelly seed and soft nose were considered as jelly seed.

Tissues sections (0.5 cm x 0.5 cm x 0.75 cm) of the peduncular extension, the interior of the mesocarp or at the distal end of the fruit were excised from both healthy and disordered fruit with a scalpel. In disordered fruit, samples were taken only from the disordered areas. Excised samples were immediately placed in glass vials containing a fixative made of 10 ml formaldehyde (Fisher F-79), 5 ml 25% glutaraldehyde (Fisher, biological grade), 1.15 g Na_2PO_3, and 0.27 g NaOH in 86 ml of deionized water (G. Erdos, IFAS, personal communication, 1995; McDowell and Trump, 1976).

Infiltration and embedding. The excised samples from the peduncular extension of the fruit and the mesocarp remained in the fixative for about 12 to 16 weeks. The samples were washed in 25% EtOH and then placed in 30% EtOH for 2 hours, then

transferred to another bath of 50% EtOH for 2 hours. To remove air from the tissue sections, the samples were then placed in a vacuum oven and the chamber evacuated with a vacuum pump until all sections sank to the bottom of the alcohol. The samples were removed from the vacuum oven, transferred to 70% tertiary butyl alcohol (TBA) for 10 hours, and transferred consecutively to 85% and 95% TBA each for 2 hours. The tissue samples were then successively transferred three times to 100% TBA for 2, 2, and 1 hours, respectively. Finally, the samples were placed in another 100% TBA bath for a 10-hour period. The samples were removed from the TBA and placed into a 1:1 mixture of TBA plus mineral oil for 2 hours. Next, the samples were successively transferred to a mixture of 1:1 mineral oil plus paraffin at 57°C for 2 hours and then to a 1:3 mixture of mineral oil plus paraffin at 57°C for 2 hours. The tissue samples were then transferred to three successive baths of 100% paraffin at 57°C for 2 hours each. Finally, using 22 mm^2 embedding molds (Shandon Lipshaw, Polysciences Inc., PA) the tissue samples were embedded in 100% paraffin (B. Dehgan, IFAS, personal communication, 1996; Johansen, 1940).

Microtomy. Embedded tissues were affixed on 2 cm x 2 cm x 2 cm wooden blocks and sectioned with a rotatory microtome to 8 to 12 μm thickness, depending on the condition of the tissue. For fruit with SEC, excised samples from the peduncular extension were sectioned both transversally and longitudinally. It was easier to cut thinner sections of healthy tissues, whereas it was necessary to use thicker sections for disordered tissues. Tissue sections were fastened to glass slides with Bissing's adhesive. The adhesive was prepared by dissolving 1 g of high grade Knox gelatin in 100 ml of deionized water at 35°C. After the gelatin was completely dissolved, 5 g of phenol and 15 ml of glycerin were added. The mixture was stirred for 5 minutes, filtered through Whatman # 1 filter paper, and allowed to cool. The preparation was combined with 3% formalin at 1:140 v:v ratio and vigorously mixed for 1 minute (T. Lucansky, IFAS, personal communication, 1995).

Staining. The paraffin was removed by placing the slides in glass staining dishes containing 100% xylene, twice for 5 minutes each time. The slides were then transferred to 1:1 xylene and EtOH for 5 minutes, successively transferred to 100%, 95%, 70%, 50%, and 30% EtOH for 5 minutes each, and stained with a 1% Safranin-O solution for 8 hours. The Safranin-O solution was prepared by dissolving 1 g of Safranin-O (Fischer Scientific, Pittsburgh, PA) in 100 ml of distilled water. The slides were removed from the safranin solution and dipped in distilled water for 5 seconds to remove most of the safranin, and then transferred to 30% EtOH for 5 minutes. Three other transfers to 50%, 70%, and 95% EtOH were successively performed, each of which lasted 5 minutes, and the slides were stained with Fast Green FCF. The Fast Green solution was prepared by dissolving 1 g of Fast Green FCF (Fischer Scientific, Pittsburgh, PA) in a mixture of 125 ml clove oil plus 125 ml 95% EtOH (B. Dehgan, IFAS, personal communication, 1995). After 20 seconds, the slides were removed from the Fast Green solution and transferred to 95% EtOH for 5 minutes. The slides were then transferred twice to 100% EtOH for 5 minutes each time. The alcohol treatment was followed by a 1:1 xylene and 100% EtOH treatment for 3 minutes. Finally, the slides were placed in three consecutive baths of 100% xylene for 3 minutes each time and mounted in Permount (Fischer Scientific, Pittsburgh, PA). A WILD MPS52 camera (Leitz, Heerburg, Switzerland) connected to a Laborlux S microscope (Leitz, Heerburg, Switzerland) at 10 to 20X magnification was used to photograph the prepared slides showing each stage of the different disorders.

Results

The time at which the first symptoms of internal breakdown appeared varied with the cultivars and with the disorders. In 1995, the symptoms of internal breakdown were first noticed 8 WAFS in 'Tommy Atkins' and 'Van Dyke', when the average fruit

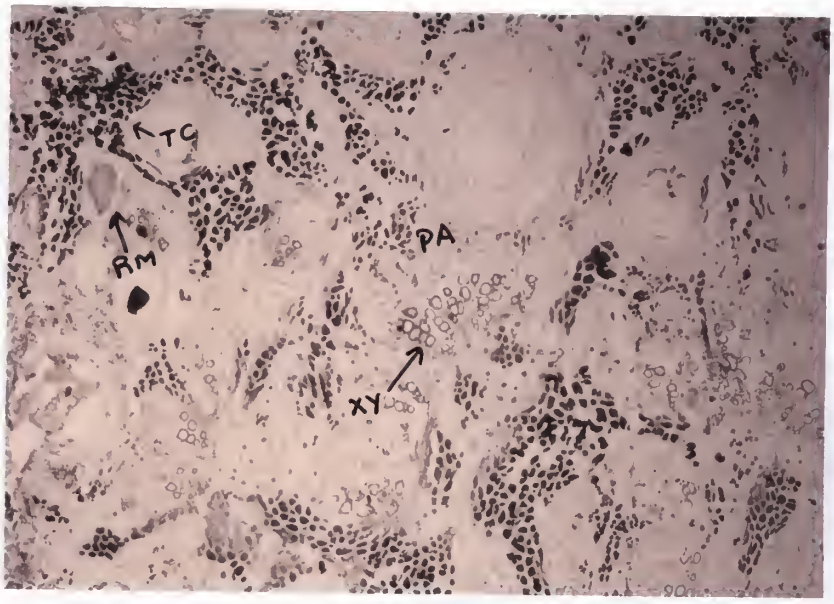
weight was approximately 17% and 10%, respectively, of the final weight. Fruit of 'Irwin' did not show the disorder until 12 WAFS, when the fruit were about 68% of their full weight. In 1996, the disorder also first appeared 8 WAFS, when the 'Tommy Atkins' were about 22% of their full weight. Early symptoms of jelly seed and SEC were observed both in immature and mature fruit, whereas soft nose was only observed in fully developed fruit.

Histology of healthy fruit. When a transverse cut was made under the skin at the peduncular extension of a healthy mango fruit, the exposed tissues appeared uniformly green or yellow, depending on the stage of maturity of the fruit. An examination of the cross section revealed that the tissues of the fruit base were maintained in close cell cohesion (Fig. 3-4). The xylem vessels were intact and undisturbed. The surrounding parenchyma also was intact, including specialized cells such as tanniferous cells or starch idioblasts. The resin ducts also appeared intact and it was possible to observe the presence of some resin masses. In longitudinal section, the cells were in perfect cohesion and no separations between tissues were observed. Resin ducts with their secretory cells and procambial strands were intact, and the presence of intact helicoidal wall thickenings of the vascular bundles was also discernible (Fig. 3-5).

When healthy fruit were cut open, the mesocarp was uniformly greenish or yellow, depending on the stage of maturity of the fruit. Microscopic examinations revealed that the mesocarp of a healthy, immature mango fruit was not very different from that of a healthy, mature fruit. In both cases, cell cohesion was observed in all areas of the mesocarp, cells were intact and contained numerous starch grains. However, more starch grains were observed in the cells of the green fruit mesocarp (Fig. 3-6) than in the cells of the mature fruit mesocarp (Fig. 3-7). Fibers with reduced wall thickening were also observed. When the healthy fruit were ripe, the cells of the mesocarp appeared empty (Fig. 3.8), i.e. the cellular content was lost but cell cohesion persisted.

Fig. 3-4. Photomicrograph of the cross section (10X) of the peduncular extension of a mango fruit free of internal breakdown. TC= tanniferous cells; RM= resin mass; PA= parenchyma; XY= xylem.

Fig. 3-5. Photomicrograph of the longitudinal section (10X) of the peduncular extension of a mango fruit free of stem-end cavity. RD= resin duct; SC= secretory cell; HWT= helicoidal wall thicknening.


TC
RM
PA
XY

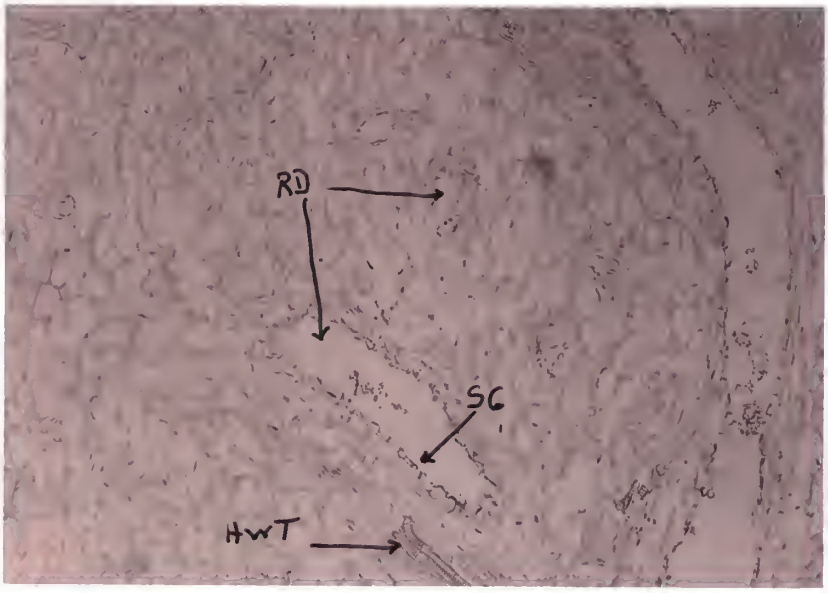
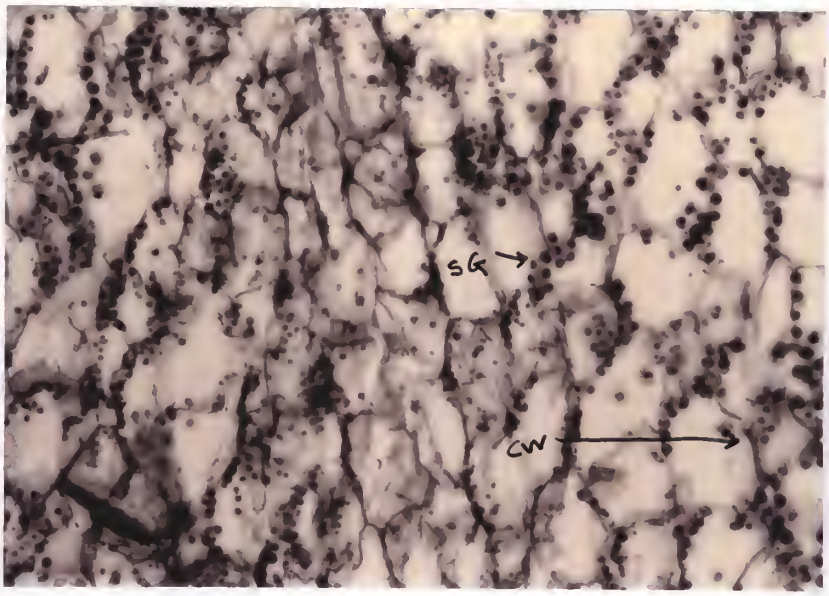
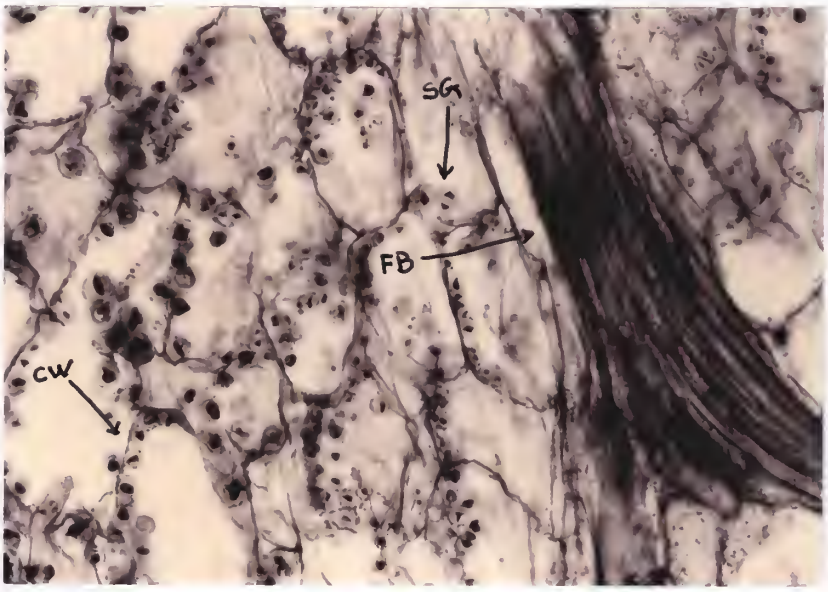

RD
SC
HwT

Fig. 3-6. Photomicrograph (20X) illustrating the mesocarp of an immature mango fruit free of internal breakdown. SG= starch grains; CW= cell wall.

Fig. 3-7. Photomicrograph (20X) illustrating the mesocarp of a mature mango fruit free of internal breakdown. CW= cell wall; SG= starch grain; FB= fiber bundle.


SG
CW


SG
FB
CW

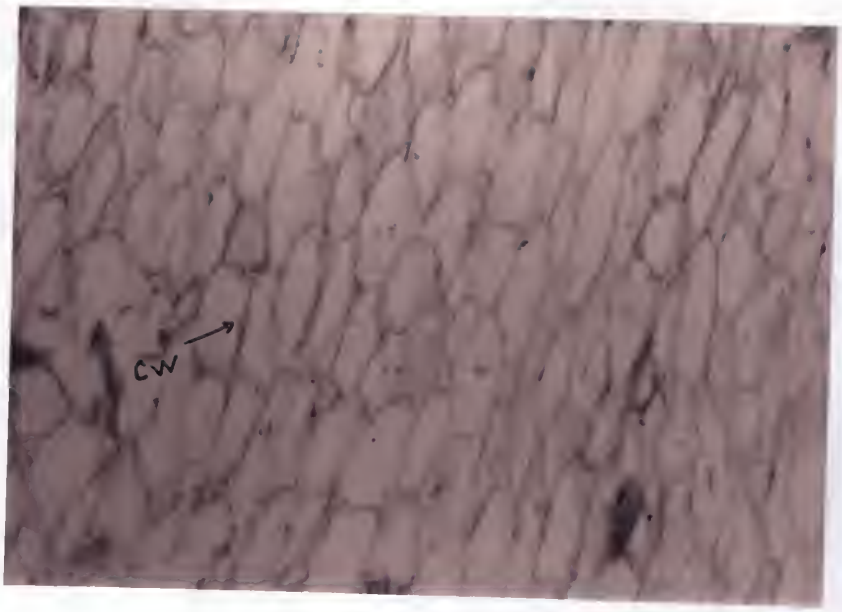
Development of stem-end cavity. The early stage of SEC did not reveal significant alterations in the peduncular extension of the fruit when the section was visually examined. After the transverse cut was made, it was possible to observe a series of brown dots circularly distributed and located at the openings of the resin ducts. The remaining tissues inside and outside the circle of brown dots appeared to be intact. Although the disorder was still at its early stage, latex exudation was less than that observed in healthy fruit, and the exuded latex had a darker color than that of healthy fruit, which was of milky appearance in both immature and mature fruit.

When a cross section of the peduncular extension of the fruit was examined with a light microscope (10 to 20X), tissues appeared partially disrupted. The disorganization mainly affected the parenchyma cells and the resin ducts in which the secretory cells had disintegrated. Little damage was apparent in the xylem, but in rare vascular bundles some tracheary elements were separated from the neighboring elements of the bundles. In the areas where the wall of the parenchyma cells were broken, the cellular contents were lost or dispersed and free tannin granules and starch grains were easily observed (Plate 3-9). A higher number of Ca oxalate crystals was also observed in sections made from tissues with SEC than in those without the disorder. In longitudinal section, areas of intact cells alternated with areas of disintegrated cells and broken cell walls. In intact areas, the cell contours were clearly defined and the cellular contents were preserved. In areas where the cells were destroyed, the cells had lost their contents, the vascular tissues were altered, and the secretory cells were absent from the inner layer of the resin ducts (Fig. 3-10).

At the intermediate stage of SEC, there was a brown discoloration at the center of the peduncular extension of the fruit; surrounding areas were often still green, and the flow of latex was greatly reduced. A cavity had formed at the proximal end of the fruit between the skin and the stone, resulting from the destruction of the vascular connections that linked the seed to the plant. The tissues around the cavity were

Fig. 3-8. Photomicrograph (10X) illustrating the mesocarp of a ripe mango fruit free of internal breakdown. Cells are in good contact and cell walls are intact. CW= cell wall.

Fig. 3-9. Cross section (20X) of the peduncular extension of a mango fruit with early stage of stem-end cavity. The tissue is almost intact in all areas. XY= xylem; DA= disrupted area; CR= crystal; TC= tanniferous cell.


CW

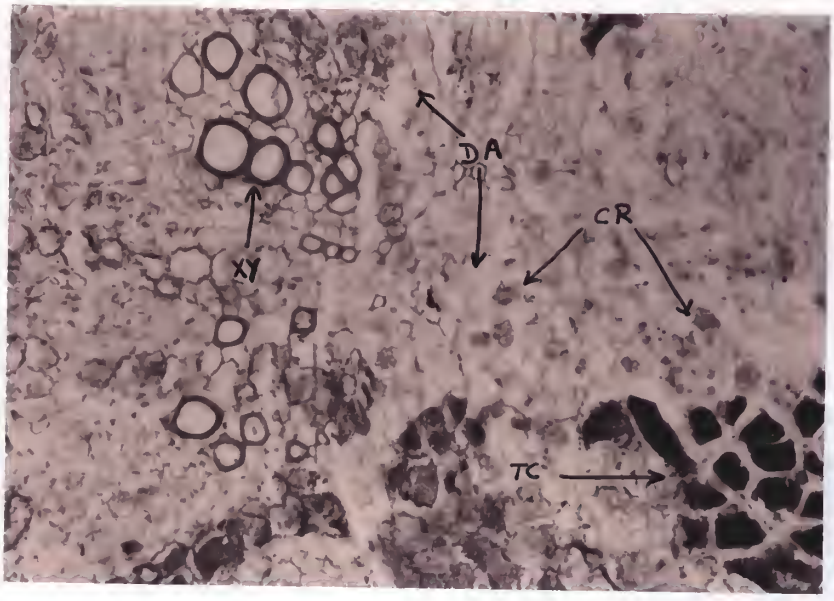
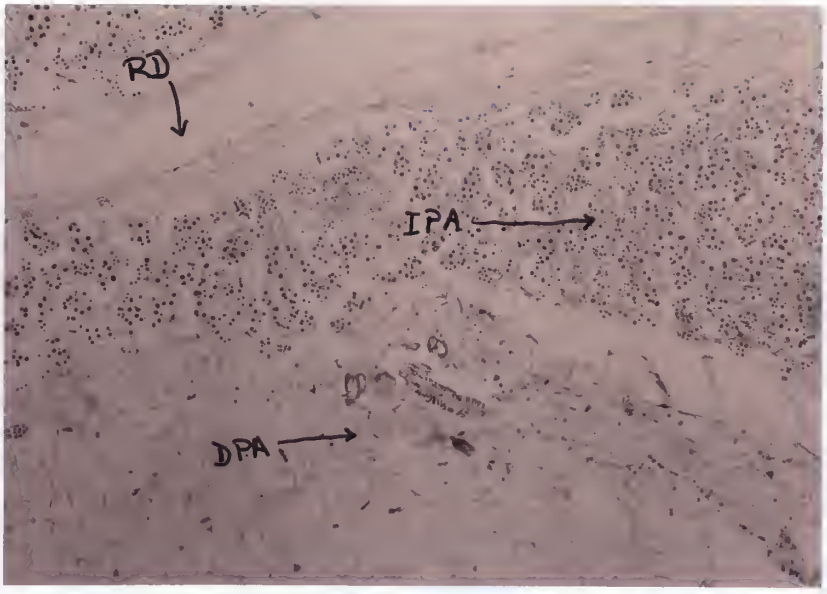
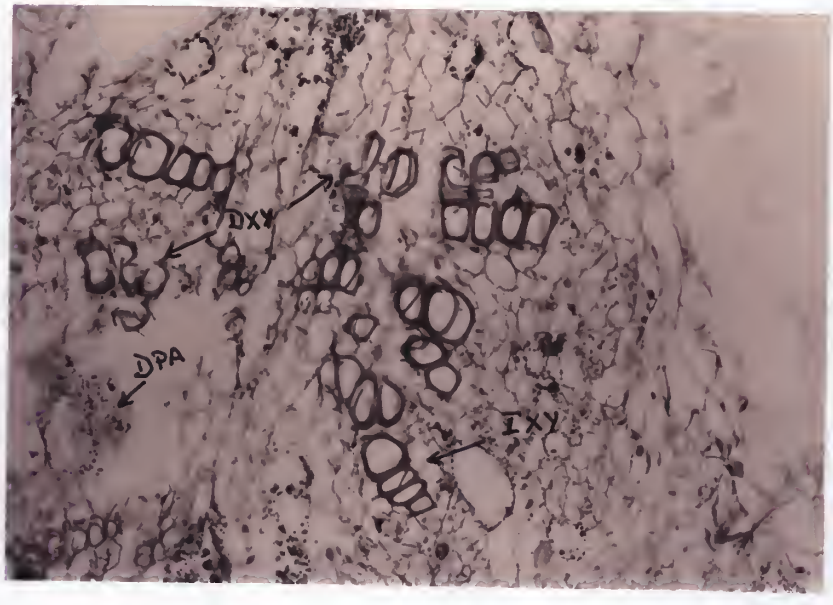

DA
CR
XY
TC

Fig. 3-10. Longitudinal section (10X) of the peduncular extension of a mango fruit with early stage of stem-end cavity. RD= resin duct; IPA= intact parenchyma; DA= destroyed parenchyma.

Fig. 3-11. Cross section (20) of the peduncular extension of a mango fruit with intermediate stage of stem-end cavity. DXY= destroyed xylem; IXY= intact xylem; DPA= destroyed parenchyma.


RD
IPA
DPA


DXY
DPA
IXY

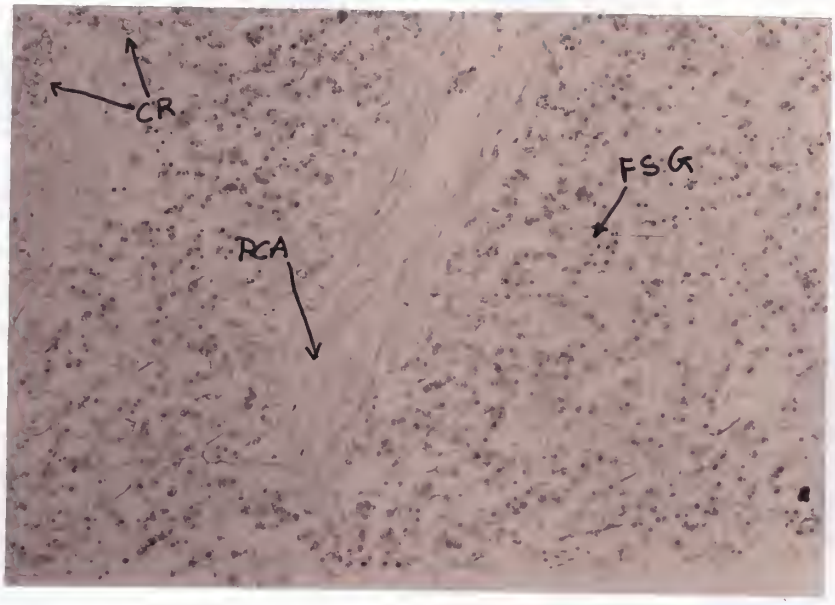
necrotic and dry, and the inner mesocarp had a yellow coloration, which was a sign of early senescence, in contrast to the outer mesocarp that remained green. At the cellular level, the damage to the parenchyma and to the vascular tissue appeared to be more extensive at the intermediate stage compared to the early stage. A cross section of the peduncular extension of the fruit revealed that large portions of the parenchyma cells and the tanniferous cells had coalesced, and resin ducts were indistinguishable. Few starch grains were present, and a great portion of the xylem from the vascular bundles was destroyed and/or destabilized (Fig. 3-11). Observations of longitudinal sections of the peduncular extension of the fruit revealed a high degree of tissue breakdown, resulting in agglomerations of cell wall fragments and free starch grains interspersed with intact cells. The procambium was partially destroyed and calcium oxalate crystals were also present. The presence of the calcium crystals and starch grains was verified with light polarizing filters (Fig. 3-12).

When the SEC disorder reached the advanced stage, the cavity was considerably enlarged compared to the intermediate stage. The seed was totally disconnected from the peduncle, and the necrosis had reached the surrounding mesocarp at the proximal end of the fruit. The interior of the mesocarp had a darker yellow coloration, but no breakdown was observed in the interior of the mesocarp around the stone. The cross section of the peduncular extension of the fruit revealed that most of the starch idioblasts were destroyed; a few tanniferous cells and tracheary elements remained. No resin ducts were distinguishable and most starch grains had disappeared (Fig. 3-13). The longitudinal section also showed a breakdown of the vascular tissues, the xylary elements had deteriorated and the procambium was destroyed (Fig. 3-14).

Development of jelly seed. When a mango fruit with early symptoms of jelly seed was cut open, the interior of the mesocarp, i.e. the portion of the mesocarp located next to the seed, had a yellow coloration, which contrasted with the surrounding greenish exterior mesocarp. The latex flowed normally from the proximal

Fig. 3-12. Longitudinal section (10X) of the peduncular extension of a mango fruit at an intermediate stage of stem-end cavity. FSG= free starch grains; CR= crystal; PCA= procambium.

Fig. 3-13. Cross section (20X) of the peduncular extension of a mango fruit with an adcanced stage of stem-end cavity. IXY= intact xylem; DPA= destroyed parenchyma; CR= crystal; DXY= destroyed xylem.


CR
FSG
RCA

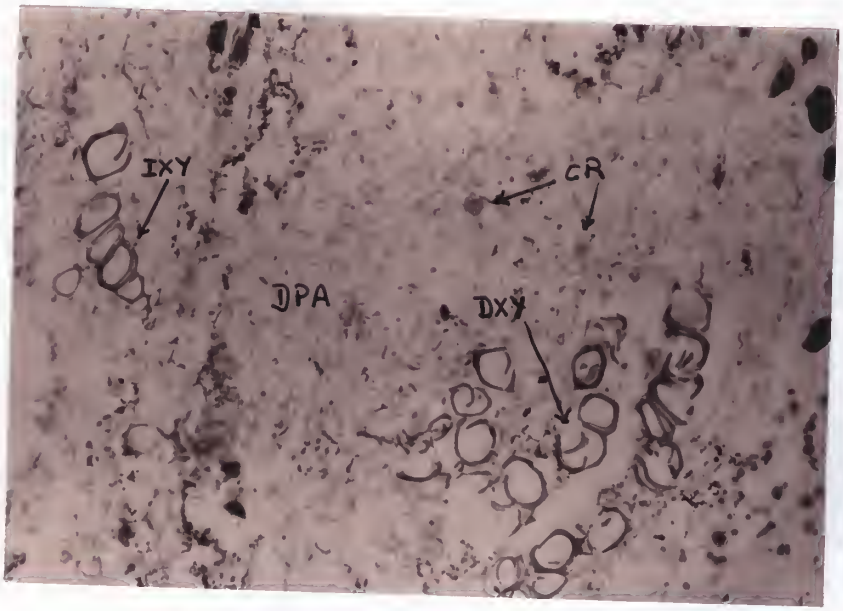
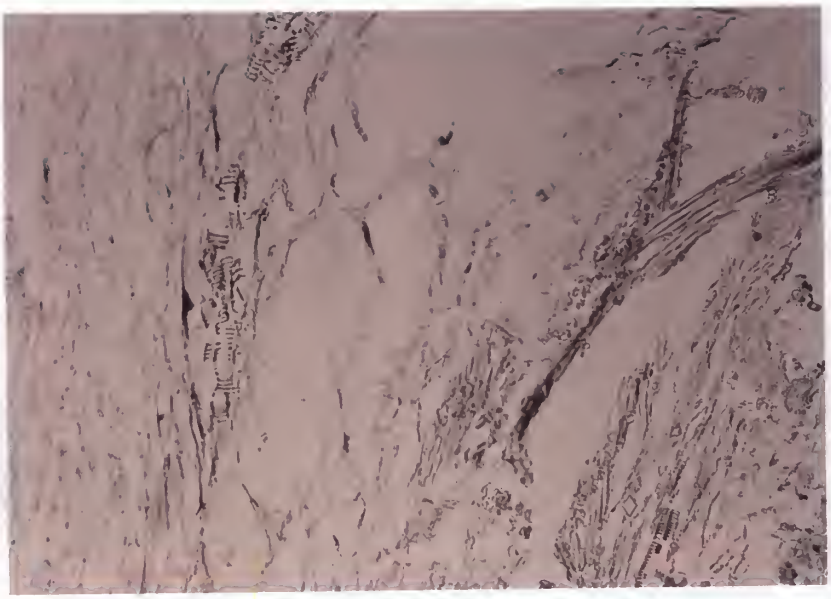

IXY
CR
DPA
DXY

Fig. 3-14. Longitudinal section (10X) of the peduncular extension of a mango fruit with an advanced stage of stem-end cavity. Damage to tissues is generalized.

Fig. 3-15. Photomicrograph (10X) of the internal mesocarp of a mango fruit in the early stage of jelly seed. Cells contain few starch grains, and walls in many cells are broken. SG= starch grains; BCW= broken cell wall; ICW= intact cell wall.



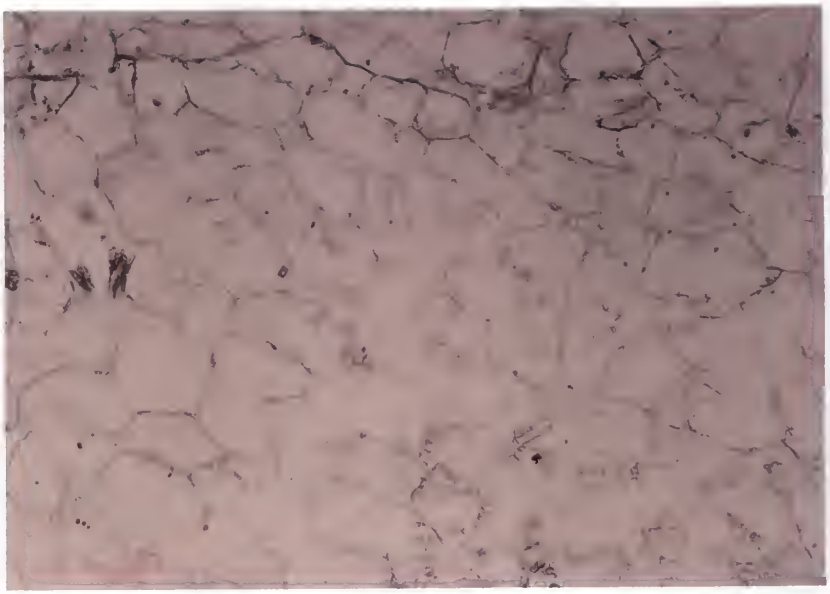

BCW
ICW
SG

end of the fruit through the resin ducts. Most cells of the disordered mesocarp appeared intact. The number of starch grains present in the cells was smaller than the number observed in the healthy mesocarp. In general, cell cohesion was maintained and the organization of the tissue was preserved (Fig. 3-15). At the intermediate stage of jelly seed, visual examinations revealed that the interior of the mesocarp had an orange coloration that was localized around the seed, whereas the exterior of the mesocarp was green or pale yellow. The affected portion of the mesocarp was softer than the non-affected portion of the fruit, and exuded juice if a slight pressure was applied. Microscopic observations revealed an extensive disintegration of walls of most cells. Also, the number of starch grains was greatly reduced compared to the number observed in the mesocarp cells at the early stage of the disorder (Fig. 3-16). The visual symptoms of the advanced stage of jelly seed consisted of softening of the interior of the mesocarp, which had the consistency of jelly or paste, and a brown coloration around the stone. Microscopically, the tissue appeared to be completely deteriorated, the walls of almost all cells were broken, large air spaces were observed, and starch grains were interspersed among the mass of broken cell walls (Fig. 3-17).

Development of soft nose. The early symptoms of soft nose were visually characterized by a dark yellow coloration of the mesocarp distal end, between the apex of the fruit and the stone apex. The remaining tissues of the fruit were intact. Unlike jelly seed and SEC, the first signs of soft nose were not observed in immature mangoes. Microscopically, cell cohesion was observed among most of the cells in the disordered mesocarp. A few cells were destroyed, and there were fewer starch grains in each cell as compared to the number observed in the cells of healthy mesocarp (Fig. 3-18). As the disorder progressed to the intermediate stage, the affected area enlarged and reached the endocarp distal end. The disordered tissue appeared softer and exuded juice with only slight pressure. Microscopic examination of a section of the mesocarp affected by soft nose at the intermediate stage revealed extensive cellular breakdown,

Fig. 3-16. Photomicrograph (10X) of the internal mesocarp of a mango fruit with an intermediate stage of jelly seed.

Fig. 3-17. Photomicrograph (20X) of the internal mesocarp of a mango fruit with an advanced stage of jelly seed. Walls of most cells are broken, starch grains are dispersed.



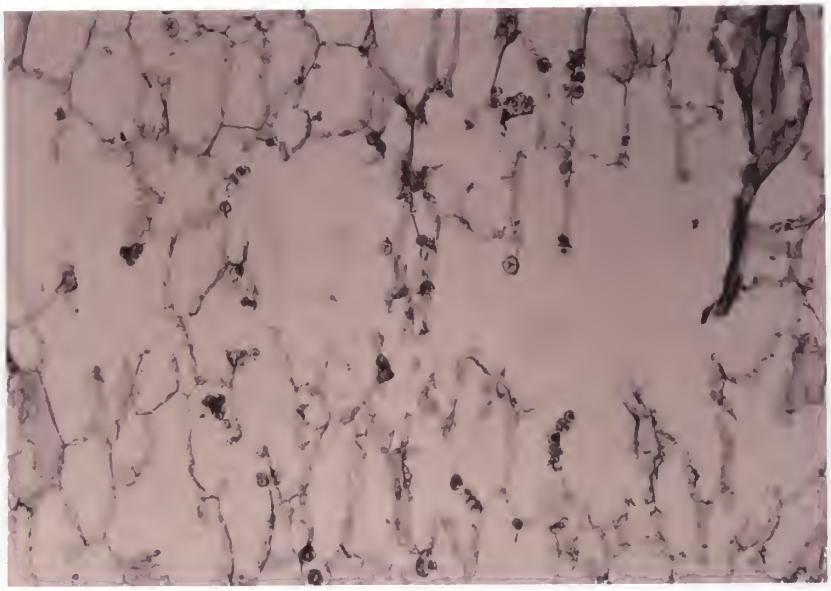
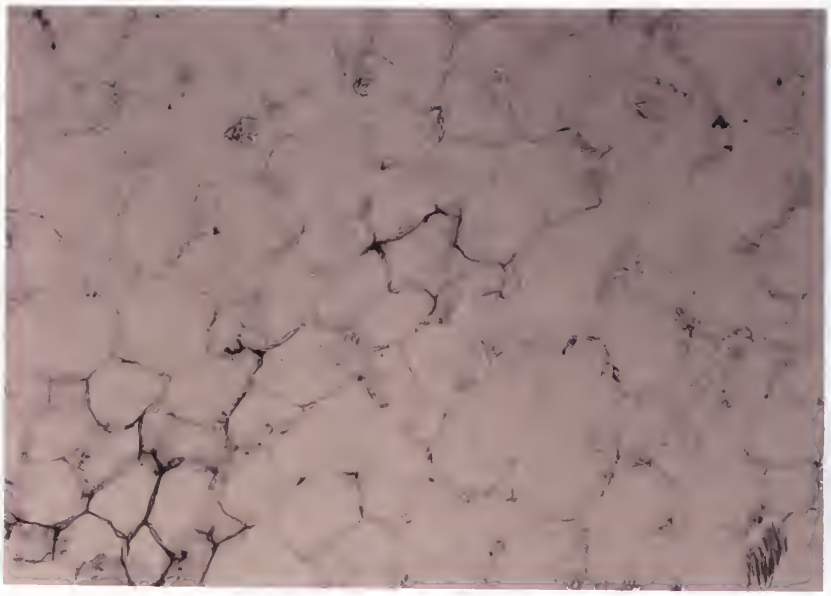
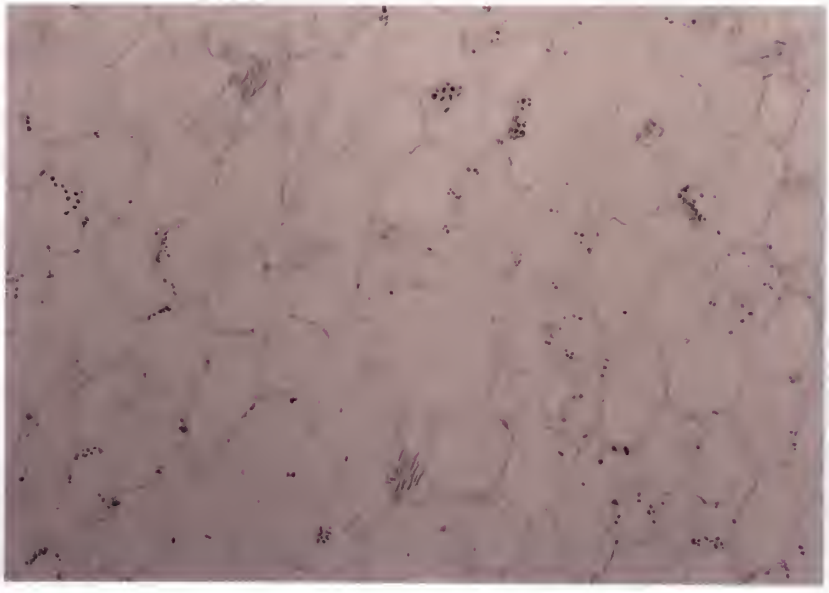


Fig. 3-18. Photomicrograph (20X) of the distal mesocarp of a mango fruit with an early stage of soft nose. Cellular content is partially lost, damage to cell walls are is reduced.

Fig. 3-19. Photomicrograph (20X) of the distal mesocarp of a mango fruit with an intermediate stage of soft nose. Damage to cell walls is more extensive. Few cells are intact.





and only a few starch grains could be discerned (Fig. 3-19). When soft nose was in an advanced stage, a large portion of the mesocarp was destroyed. The affected area included the sinus or beak of the fruit, and had reached the concave portion of the stone. Unlike jelly seed, the breakdown did not entirely surround the endocarp. At this stage, the disordered tissue had the consistency of paste, and there was a lack of firmness at the distal end of the fruit as compared to the firmness of a healthy, ripe fruit. The reduced firmness at the distal end of the fruit could be used to accurately diagnose the presence of soft nose without cutting open the fruit. All attempts to prepare slides from the mesocarp of a mango fruit exhibiting an advanced stage of soft nose failed.

Discussion

There are macroscopic differences among jelly seed, soft nose, and SEC. Symptoms of each type of disorder are different when the disorder first develops and each disorder affects different areas of the fruit. SEC affects the proximal end of the fruit and is characterized by destruction of the vascular tissues of the peduncular extension of the fruit. The symptoms of SEC as described in the literature are mostly based on observations of ripe fruit (Galán-Sauco et al., 1984; Winston, 1984). However, in the present study, it was observed that the cavity was formed before the fruit were fully developed. Similar observations were reported by Malo and Campbell (1978), who indicated that flesh breakdown may start before fruit are fully mature. A brown coloration was observed at the openings of the resin ducts at the early stage of SEC. Deposits of tannins in the resin ducts and in the xylem elements of fruit affected by black tip necrosis have been reported (Das-Gupta and Asthana, 1944). In that study, which involved the maceration of the fruit stalk in weak alcali solution, the deposits were found in discontinuous, viscous masses scattered throughout the duct system. The

resin ducts appeared to contain larger masses of tannins than the xylem vessels (Das-Gupta and Asthana, 1944). There is a possibility that tannins were involved in the brown coloration of the resin ducts of fruit proximal end sections with SEC observed in the present study. Entire resin ducts or xylem vessels could not be individually examined. However, it is possible that the accumulation of resin in the ducts and in the vessels could have reached levels that were toxic to the tissues and caused the necrosis observed in the intermediate and advanced stages of SEC.

More Ca oxalate crystals were observed in cross sections of the peduncular extensions of fruit with SEC than in healthy fruit. It is possible that Ca accumulates as crystals in the cytoplasm. While in the crystalline form, Ca may not be available for biochemical functions. Accumulation of Ca in the crystalline form may induce a localized deficiency within the fruit, although the disordered fruit as a whole may contain similar Ca concentrations as the healthy fruit.

Jelly seed has been considered to be the ultimate stage of SEC (Winston, 1984), and has been included among the symptoms of flesh breakdown (Malo and Campbell, 1978). However, in this study, jelly seed symptoms first appeared at the same time as SEC symptoms, when the fruit were still immature. Soft nose has been described as a combination of jelly seed and SEC (Galán-Sauco, 1984). The present study shows that there are differences among SEC, jelly seed, and soft nose. Jelly seed affected only the interior of the mesocarp, whereas SEC affected the proximal end of the fruit. Soft nose symptoms first appeared when the fruit were close to maturity and, although the disorder may affect a large portion of the mesocarp in the advanced stage, soft nose mostly affected the fruit mesocarp distal end. The symptoms of jelly seed developed all around the endocarp, whereas those of SEC may also affect the interior mesocarp entirely or partially. SEC caused a deterioration of the vascular tissues between the stone and the mesocarp, and a necrosis of the mesocarp around the cavity. However, no cavity, air pocket, gap or tissue necrosis developed in fruit affected with jelly seed

or soft nose. Consequently, the premature senescence of the mesocarp observed in SEC, jelly seed, and soft nose may not be caused by the same factors.

Although the visual symptoms of the different types of disorders appeared different, no major microscopic differences were established among jelly seed, soft nose, and SEC. In all three disorders, the disorganization of the cells and rupture of the cell walls appeared as the first microscopic indicators of all these disorders. Once the cell walls were disrupted, the cellular contents were released and, as time passed, the disorders extended to areas that were intact until the affected tissues became watery, pasty, or jelly-like.

In this study, it was not possible to investigate in detail the alterations caused to the cell walls. The available literature does not contain information about the biochemical changes associated with jelly seed, soft nose, or SEC in the mesocarp of the mango fruit. However, research on other physiological disorders indicates that significant changes occur in the activities of several enzymes present in the mesocarp of disordered fruit. The activities of malic enzyme and pectin methylesterase increased in the mesocarp of 'Alphonso' mangoes affected with spongy tissue (Krishnamurthy, 1981). Also, Katrodia (1988) indicated that amylase activity was three times lower (1.66 mg maltose/hr/mg protein at 37°C) in mesocarp with spongy tissue than in healthy pulp (4.13 mg maltose/hr/mg protein at 37°C). Additionally, Katrodia et al. (1988) reported that invertase activity was significantly lower in the mesocarp of fruit affected with spongy tissue (0.46mg hexose/hr/mg protein at 37°C) than in healthy ripe mesocarp (1.79 hexose/hr/mg protein at 37°C). Spongy tissue is different from jelly seed, soft nose, and SEC. However, as observed in fruit with spongy tissue, the breakdown observed in jelly seed, soft nose, and SEC was probably the result of enzymatic activities.

Tissues with jelly seed and soft nose symptoms contained fewer fiber strands than healthy mesocarp. This may have been caused by a destruction or dissolution of

the fibers early in the onset of the disorders. Similar deterioration of the vascular tissues was observed in SEC. The deterioration of fibers and other tracheary elements may have isolated the rest of the mesocarp from the seed, interrupting the supply of nutrients to the mesocarp.

Conclusions

Anatomical observations indicated that jelly seed, soft nose, and SEC are different disorders. Observations during the early stages of fruit ontogeny allowed the disorders to be distinguished at their earliest stages of development. SEC and jelly seed disorders affect mango during early fruit development, while soft nose developed when the fruit were nearly mature. Consequently, it would be impossible to reverse the breakdown process once it is initiated. Therefore, control measures should be applied at an early stage of fruit ontogeny to prevent or minimize internal breakdown in mango. It was not possible with light microscopy to determine in detail the type of alterations caused to the cell walls. Attempts to localize Ca in the cell walls through scanning electron microscopy or histochemical Ca tests were unsuccessful. Deterioration or dissolution of vascular connections between the stone and the mesocarp was also a common feature among the three disorders. The premature senescence of the disordered tissues may have resulted from the interrupted transport of photoassimilates due to the destruction of the vascular connections between the sites of the disorders and the stone. There were no reports on the presence of Ca crystals in the stalk end of mango fruit before this study. If internal breakdown is effectively due to Ca deficiency, the deficiency could be caused by formation of Ca crystals resulting in an unavailability of Ca for normal cell functioning.

CHAPTER IV

MINERAL ELEMENT CONCENTRATIONS IN MANGO (*Mangifera indica* L.) LEAVES AND FRUIT THROUGHOUT FRUIT ONTOGENY

Introduction

Fruit of several crop species are affected by a variety of disorders considered to be due to nutritional imbalances or mineral deficiencies. Among these disorders are 'bitter pit' of apple, 'cork spot' of pear, 'translucent flesh' of mangosteen, 'blossom-end rot' of tomato, 'blackheart' of celery, 'internal breakdown' of mango, and 'glassiness' of melon. These disorders are attributed to Ca deficiency (Bangerth, 1979; Odet and Dumoulin, 1993; Shear, 1975). Internal breakdown, a physiological disorder that affects fruit of several commercially grown mango cultivars, can cause losses of up to 80% of an annual crop (Iyer and Subramanyan, 1992). The disorder is presumed to be due to Ca deficiency in the fruit (Young, 1957). However, it may also be due to an imbalance of other nutrient elements in the fruit. Studies of other fruit crops indicated that mineral element concentrations in fruit and leaf tissues change with time. Drake et al. (1974) observed increased leaf Ca concentrations during apple fruit development, with values of 0.35% in June, 0.83% in July, and 0.99% in August. Chiu et al. (1976) correlated increased incidence of blossom-end rot in tomato with Ca deficiency during the fruiting period. Bernadac et al. (1996) investigated the changes in fruit calcium concentrations during development of hydroponically grown melon (*Cucumis melo* L.) plants by the nutrient film technique (NFT). Fruit Ca concentration decreased with time after withholding Ca and 3 to 9-day-old fruit were most vulnerable to the Ca-deficiency

treatment. In fruit that were 10 or more days old, there were no significant effects of withholding Ca on fruit Ca concentration.

Previous reports on internal breakdown in mango fruit do not contain detailed information on leaf and fruit mineral composition throughout fruit ontogeny. Mineral concentrations in plant tissues may vary according to environmental factors, genetics, or cultural practices. These factors may indirectly affect the incidence of disorders by affecting mineral element concentrations in the fruit. Burdon et al. (1991) studied the mineral content of immature and mature green 'Kent' and 'Beverly' mangoes. The cultivar Kent is considered to be susceptible to the soft-nose disorder whereas 'Beverly' is not. Fruit were collected from two sites 12 Km apart. At site I, fruit were prone to the soft-nose disorder and at site II, fruit were not affected by the disorder. Burdon et al. (1991) observed that the Ca concentration of fruit from site I was lower than of fruit from site II. There were no differences in fruit Ca concentration between 'Kent' and 'Beverly' mangoes grown at site I. Young and Koo (1969) determined the N, P, K, Ca, and Mg concentrations of mango leaves for 10 years in Florida. Their study included 'Kent', 'Parvin', 'Haden', and 'Zill' mangoes grown on Lakewood soil (acidic soil), and 'Kent' and 'Haden' mangoes grown on Rockdale soil (alkaline limestone). Calcium concentrations in the leaves were higher in alkaline soil, whereas leaf Mg concentrations were higher in acidic soil. Leaves from 'Kent' trees grown on Lakewood soil contained higher Ca concentrations than 'Haden', 'Parvin', or 'Zill' leaves from the same location. Avilán (1971) investigated the fluctuations of N, P, K, and Ca in leaves of 14-year-old 'Kent' mango trees in Venezuela. Trees received four $N\text{-}P_2O_5\text{-}K_2O$ fertilizer treatments including 0-0-0, 0-30-30, 40-30-30, and 80-30-30 Kg/ha. Foliar N, P, and K increased with increased fertilizer rates. However, the leaf Ca concentration was reduced from 2.56% in the 0-0-0 treatment to 2.42% in the 80-30-30 treatment.

Fruit are rapidly growing organs that require nutrients to be constantly available in adequate concentrations for normal growth. A nutritional imbalance can be established at any time during fruit ontogeny. A study of the nutritional status of mango leaves and fruit throughout fruit development may provide an understanding of the nutritional requirements and changes occurring in these tissues during fruit ontogeny. In addition, knowledge of nutritional fluctuations may enable the moment to be pinpointed at which fruit become vulnerable to nutrient-related disorders. This may allow for the determination of critical application times of mineral elements to control these disorders. This study was conducted to investigate the fluctuations in the mineral composition of mango leaves and fruit throughout fruit ontogeny, beginning 4 weeks after fruit set (WAFS) until fruit were ripe.

Materials and Methods

Location. Experiments were conducted in commercial orchards in south Dade County (25.36°N latitude and 80.21°W longitude). The soil type belonged to the Krome series (loamy-skeletal, carbonatic, hyperthermic Lithic Udorthents). These soils are 0-27.50 cm deep, and made of very gravelly loam or weathered bedrock. They contain 15-20% clay and have a pH of approximately 7.4-8.4 (USDA, 1996). Data collection began 5 weeks after fruit set in 1994 (WAFS) and 4 WAFS in 1995 and 1996, and lasted until fruit were ripe. Fruit were considered ripe when the mesocarp was soft enough for consumption as fresh fruit. This occurred in each cultivar between 15 to 18 WAFS. The daytime temperature during the experimental period averaged 25.1°C, 24.3°C, and 23.6°C in 1994, 1995, and 1996, respectively. The total amounts of rainfall were 698.5 mm, 911.0 mm, and 894.5 mm in 1994, 1995 and 1996, respectively. Rainfall was highest in April in 1994, whereas the peak was observed in June during 1995 and 1996.

Plant materials. Fruit and leaves were collected from 'Irwin', 'Tommy Atkins', and 'Van Dyke' mango trees (*Mangifera indica* L.) in three commercial orchards. Orchards I and II were used in 1994 and 1995, whereas orchard III was used in 1996. Orchard I was comprised of 15-year-old 'Van Dyke' trees, whereas orchard II included 40-year old 'Irwin' and 'Tommy Atkins' trees. In orchard III, the 'Tommy Atkins' trees were 30 years old. Trees used in this study were grafted on 'Turpentine' rootstock.

In 1994 and 1995, 'Van Dyke' trees in orchard I received 630 Kg/ha of 3-8-12 on September 15, and three applications of 5.23 Kg/ha, 7.61 Kg/ha, and 9.55 Kg/ha of Sequestrene-Fe (Geigy-138, 5% Fe) in September, January, and May, respectively. Another application of 646.59 Kg/ha of 6-0-19 was made in April 1995. In 1995 and 1996, 'Irwin' and 'Tommy Atkins' trees in orchard II received only 568 Kg/ha of 6-0-19 applied as a bulk soil treatment in April, as the orchard was scheduled to be sold.. In 1995, 'Tommy Atkins' trees in orchard III were given 568 Kg/ha of 6-0-19 in March, 9.94 Kg/ha of Sequestrene-Fe (Geigy 138, 5% Fe) in August, 852.27 Kg/ha of 3-8-12 and 19.32 Kg/ha of Sequestrene-Fe (Geigy 138, 5% Fe) in September, and 19.32 Kg/ha of Sequestrene-Zn (Geigy 138, 14% Zn) plus 19.32 Kg/ha of Sequestrene-Mn (Geigy 138, 12% Mn) in December. In 1996, no fertilizer was applied to orchard III.

Leaf and fruit samples were collected biweekly beginning 4 WAFS. Five mature, healthy leaves were collected at each sampling date from the middle portion of a fruit-bearing branch. Five trees of each cultivar and five fruit from each tree were collected during each sampling date in 1994 but in 1995 and 1996, three fruit from each tree were collected. In the 1996 study, which included only 'Tommy Atkins' trees, leaf and fruit samples were collected every week. Samples were placed in paper bags and labeled according to dates and cultivars. Fruit and leaf samples were shaded to

prevent major water loss due to prolonged exposure to sunlight during sampling and transportation from the field to the laboratory.

Fruit and leaves were washed in a 1 ml/L detergent solution, rinsed in tap water, then in 0.12 N HCl, and rinsed twice in deionized water, as described by Schaffer et al. (1988). Leaf samples were oven dried at 70°C for 48 hours. Fruit samples were dried for 48-120 hours, depending on fruit size. Dried tissue samples were ground with a cyclone mill (UDY Corp., Ft. Collins, CO). For K, P, Ca, Mg, Zn, Cu, Mn, Fe, and B determination, 1 g of ground tissue was weighed in a 40-ml High-Form porcelain crucible (Fisher Scientific Co., Pittsburgh, PA) and ashed at 500°C in a muffle furnace (Furnatrol FA1730, Barnstead/Thermolyne, Dubuque, IA). After cooling, the ashed sample was digested with 5 ml of 12.1 N HCL (Fisher Scientific, certified ACS Plus) and brought to 50 ml with deionized water. The preparation was shaken and filtered through Whatman #1 filter paper into a 20-ml polyethylene scintillation vial. Polyethylene vials were used rather than glass so that there was no borosilicate to interfere with B determinations. For N determinations, 0.2 g of ground tissue was weighed into a 100-ml digestion tube to which 2 g of Kjeldahl mixture and 5 ml of concentrated H_2SO_4 were added. Glass funnels were placed on the tubes and these were placed on a preheated aluminum block digester at 250°C for 1 hour. Temperature was then increased to 380°C for an additional 3 hours. After cooling, 5 ml of deionized water were added to each tube, and the tubes were agitated with a vortex mixer. The content of each tube was transferred to a 100-ml volumetric flask, and brought to 100 ml with deionized water and allowed to cool. The digested material was vigorously mixed, then filtered through Whatman #1 filter paper into a 20-ml polyethylene scintillation vial (Hanlon et al. 1994). Nitrogen concentrations in fruit and leaves were determined by the Total Kjeldahl Nitrogen (TKN) method, while K, P, Ca, Mg, Zn, Cu, Mn, Fe, and B were determined by inductively coupled argon plasma spectroscopy (ICAP) (Hanlon et al.,1994).

Results

Nitrogen. Leaf N concentrations remained fairly constant throughout fruit development (Fig. 4-1A-G). Fruit N concentrations decreased from 4 WAFS to fruit maturity in all cultivars during the 3 years of study. On the first sampling date, the N ratio between leaves and fruit was >1 in all three cultivars. This ratio quickly changed to <1 within 2 to 3 weeks. A period of rapid reduction of fruit N was observed between 4 to 8 WAFS. The rate of reduction in fruit N concentrations was different among cultivars. In 'Irwin' fruit, N concentration decreased at a faster rate than in 'Tommy Atkins' and 'Van Dyke' fruit. For example, in 1994, between 5 and 9 WAFS N concentrations declined 56.72%, 36.64%, and 42.40% in 'Irwin', 'Tommy Atkins', and 'Van Dyke', respectively (Fig. 4-1A-C). At the end of the sampling period, no differences in fruit N concentrations existed among the cultivars. A comparison of N concentrations observed at 4 and 14 WAFS in 1995 showed decline in fruit N concentrations of 52.34%, 67.54%, and 58.18% in 'Irwin', 'Tommy Atkins', and 'Van Dyke', respectively.

Phosphorus. Phosphorus concentrations in leaves varied little throughout fruit ontogeny (Fig. 4-2A-G). Leaves of 'Van Dyke' contained higher P concentrations than 'Tommy Atkins' or 'Irwin' leaves. In 'Tommy Atkins,' fruit P concentrations were consistently higher than foliar P concentrations throughout fruit ontogeny (Fig. 4-2B, 4-2E, 4-2G). Fruit P concentrations significantly decreased from 4 to 10 WAFS in all 3 years. The rate of P reduction in the fruit varied among cultivars. Ten WAFS in 1994, P levels in 'Irwin' fruit represented 45% of the concentration recorded at 6 WAFS. At the same time, P concentrations in 'Tommy Atkins' and 'Van Dyke' fruit, were 68% and 57.89%, respectively, of the P concentrations found in the first samples. In 1995, the declines were 60.73%, 62.22%, and 55.90% in the respective cultivars. As the fruit developed, the fruit to leaf P ratio decreased. When the fruit were ripe, the fruit P

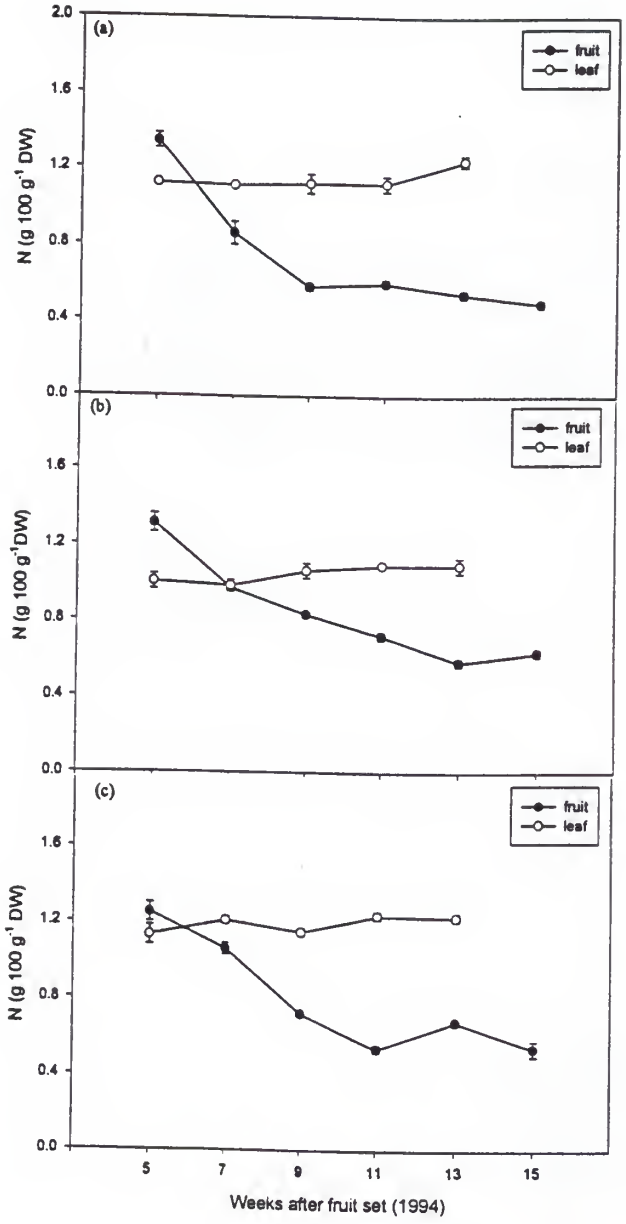
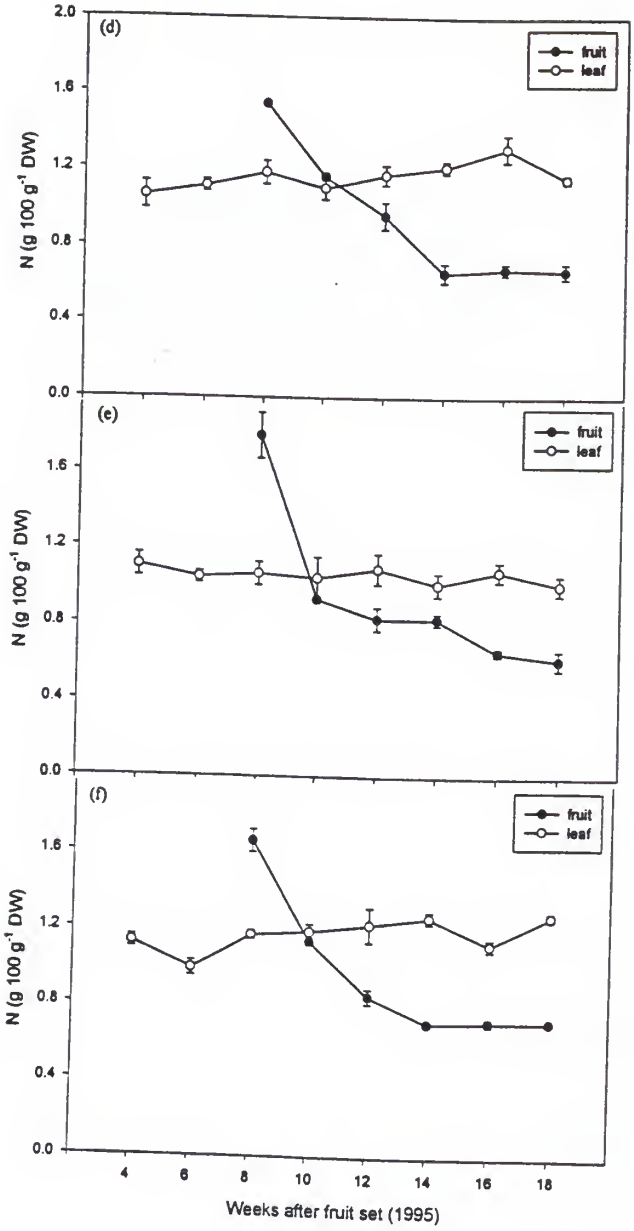
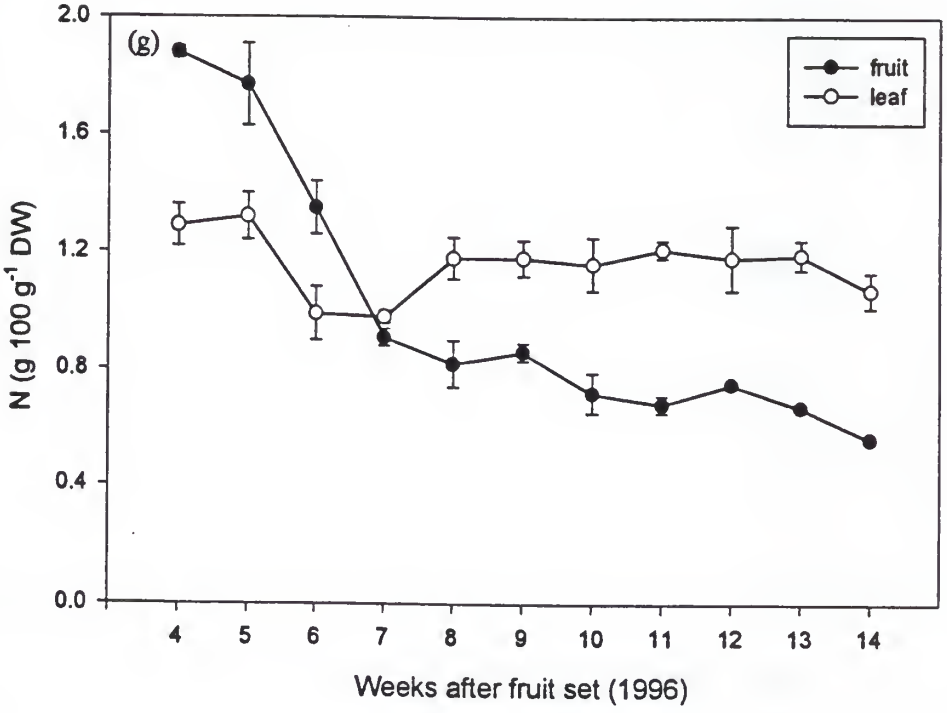


Fig.4-1. Fluctuations in the N concentrations in 1994 in leaves and fruit of 'Irwin' (a), 'Tommy Atkins' (b), and 'Van Dyke' (c), in 1995 in leaves and fruit of 'Irwin' (d), 'Tommy Atkins' (e), and 'Van Dyke' (f), and in 1996 in leaves and fruit of 'Tommy Atkins'(g), during fruit ontogeny. Values represent means of 25 fruit or 25 mature leaves. Vertical bars represent ± 1 standard error. Absence of error bars indicates that the standard error was smaller than the symbol for the mean.


(d)
(e)
(f)
fruit
leaf
N (g 100 g-1 DW)
0.0
0.4
0.8
1.2
1.6
2.0
4
6
8
10
12
14
16
18
Weeks after fruit set (1995)


(g)
fruit
leaf
N (g 100 g-1 DW)
2.0
1.6
1.2
0.8
0.4
0.0
4
5
6
7
8
9
10
11
12
13
14
Weeks after fruit set (1996)

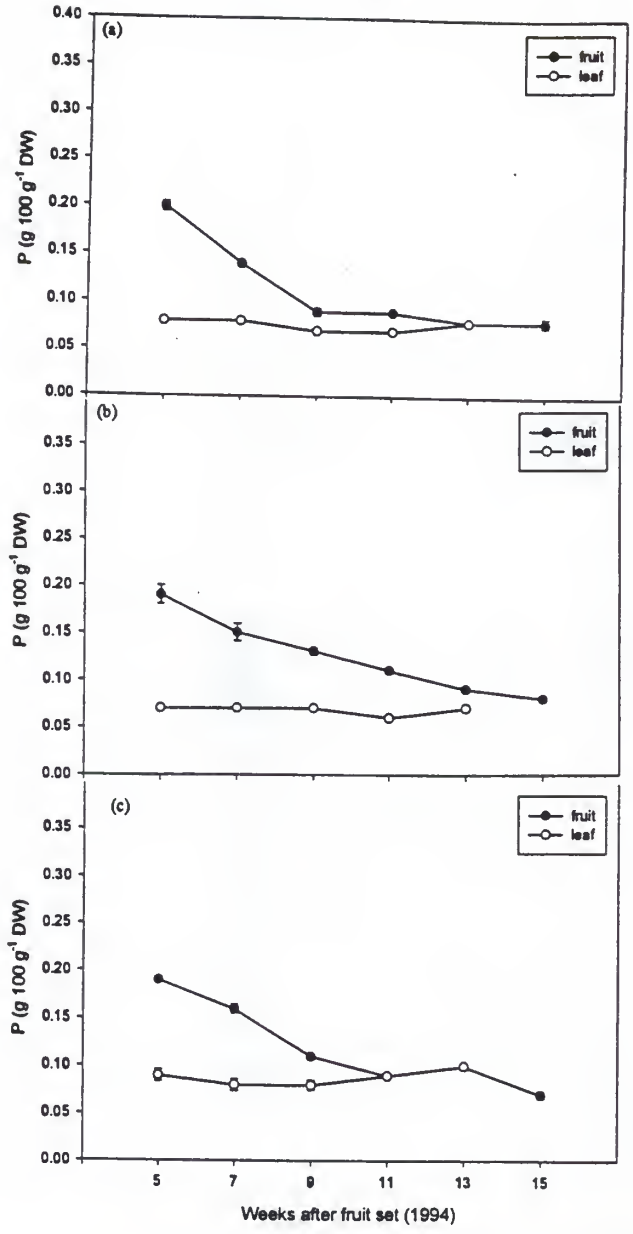
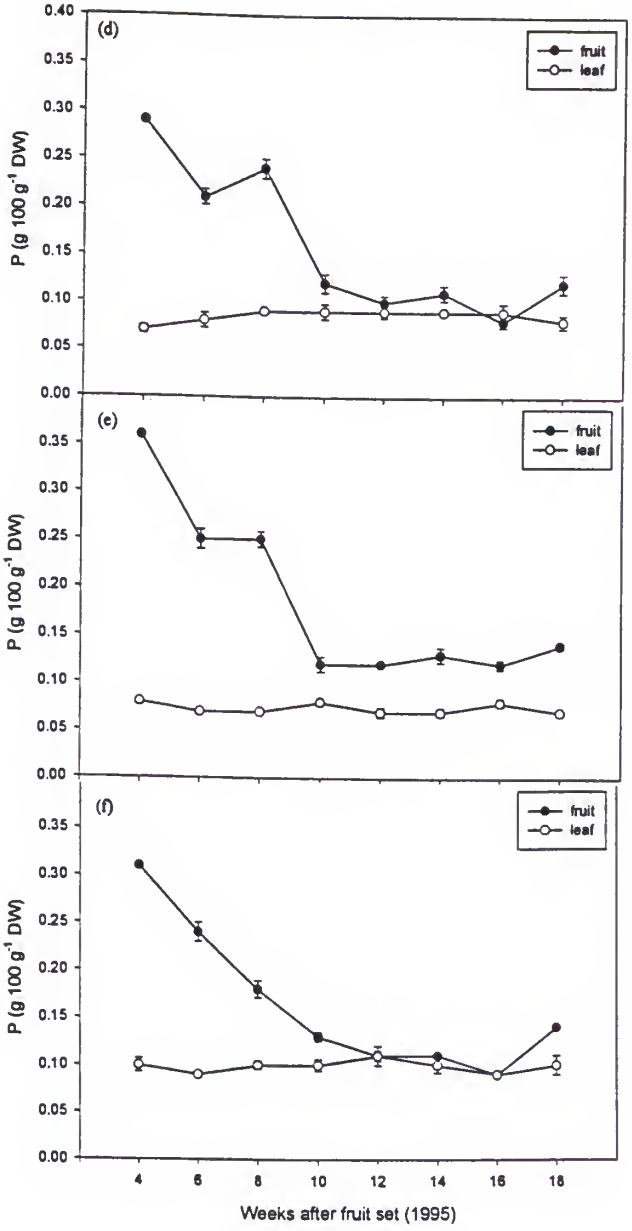
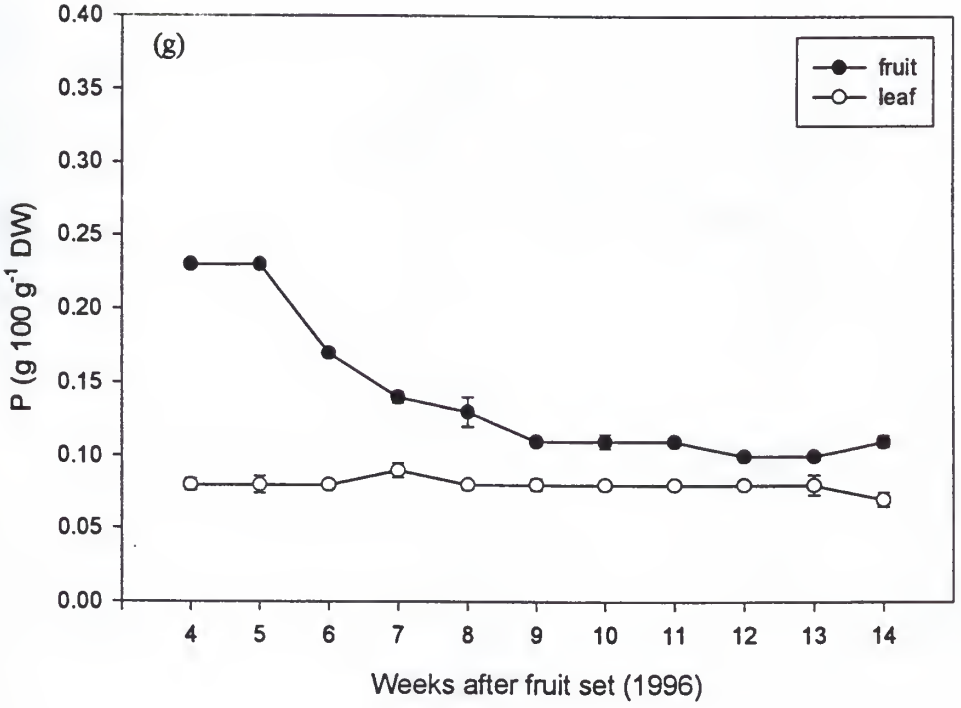


Fig. 4-2. Fluctuations in the P concentrations in 1994 in leaves and fruit of 'Irwin' (a), 'Tommy Atkins' (b), and 'Van Dyke' (c), in 1995 in leaves and fruit of 'Irwin' (d), 'Tommy Atkins' (e), and 'Van Dyke' (f), and in 1996 in leaves and fruit of 'Tommy Atkins' (g), during fruit ontogeny. Values represent means of 25 fruit or 25 mature leaves. Vertical bars represent ± 1 standard error. Absence of error bars indicates that the standard error was smaller than the symbol for the mean.


(d)
(e)
(f)
fruit
leaf
P (g 100 g-1 DW)
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
4
6
8
10
12
14
16
18
Weeks after fruit set (1995)


(g)
fruit
leaf
P (g 100 g-1 DW)
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
4
5
6
7
8
9
10
11
12
13
14
Weeks after fruit set (1996)

concentration had decreased to almost one-third of the concentration observed on the first sampling date. Ripe 'Irwin' fruit had a lower P concentration than ripe 'Tommy Atkins' or 'Van Dyke' fruit. In 1996, the P concentration was constant throughout most of the fruit development period in the leaves of 'Tommy Atkins' trees, but a significant decline was observed in the foliar P concentration when the fruit were ripe. Fruit P concentration remained constant between 4 and 5 WAFS in 1996. Between 5 and 9 WAFS, the fruit P concentration declined 48% and remained fairly constant from 9 WAFS until the fruit ripened.

Potassium. In 'Tommy Atkins', leaf K concentrations fluctuated little (Fig. 4-3B, 4-3E, 4-3G). In fruit of all three cultivars, K concentrations remained higher than leaf K concentrations throughout fruit ontogeny. Potassium concentrations in leaves of 'Van Dyke' were similar to those in leaves of 'Irwin' trees. In 1994, K concentrations in leaves of 'Van Dyke' and 'Irwin' were significantly lower at 10 WAFS than K concentrations recorded 6 WAFS (Fig. 4-3A, 4-3C). The lowest fruit K concentrations were observed in 8-week-old fruit of 'Irwin'. In 'Tommy Atkins' and 'Van Dyke' fruit, the lowest K concentrations were observed in 12-week-old and in 10-week-old fruit, respectively.

In 1995, between 4 and 8 WAFS, no significant fluctuations were observed in the leaf K concentrations of 'Irwin' trees. Leaf K concentration was highest in 'Irwin' at 10 WAFS and lowest 16 WAFS. Fruit K concentration in 'Irwin' significantly decreased 6 WAFS and increased again 8 WAFS to the concentration observed at 4 WAFS (Fig. 4-3D). Between 8 and 10 WAFS, the fruit K concentration remained constant before declining to its lowest concentration at 12 WAFS. Another increase in the K concentration was observed again in 'Irwin' fruit at 14 WAFS followed by a significant decline at 16 WAFS. When the 'Irwin' fruit were ripe (18 WAFS), the K concentration had increased 39% from the concentrations observed 16 WAFS (Fig. 4-3D). Potassium concentration in the leaves of 'Tommy Atkins' trees was constant

throughout fruit ontogeny. Fruit K concentration in 'Tommy Atkins' continuously declined from 4 to 10 WAFS (64.9%). No significant changes were observed in the fruit K between 10 and 12 WAFS. Fruit K concentration in 'Tommy Atkins' increased again 14 WAFS and did not significantly change from that time until the fruit ripened. In 'Tommy Atkins', K concentrations were higher in young fruit than they were in ripe fruit (Fig. 4-3E). Between 4 and 14 WAFS, no significant changes were observed in the leaf K concentrations of 'Van Dyke' trees. Like in 'Irwin' trees, leaf K concentrations in 'Van Dyke' were lowest at 14 WAFS. Fruit K concentrations in 'Van Dyke' were lowest at 6 and 16 WAFS, and highest in the ripe fruit. From 6 to 8 WAFS, the fruit K concentration in 'Van Dyke' had increased 18%, but the fruit K concentration continuously declined again until 16 WAFS. Potassium concentration in young 'Tommy Atkins' fruit was higher than in young 'Irwin' or 'Van Dyke' fruit. When the fruit were ripe, there were no significant differences in fruit K concentrations among 'Irwin', 'Tommy Atkins', and 'Van Dyke' (Fig. 4-3F).

In 1996, the leaf K concentration in 'Tommy Atkins' remained fairly constant throughout fruit ontogeny except for a significant decline observed at 10 WAFS. The fruit K concentration did not significantly fluctuate between 4 and 5 WAFS. However, between 5 and 8 WAFS, the fruit K concentration had declined 67.7%. That period of decline was followed by a significant increase at 10 WAFS. No significant differences existed in fruit K concentration between 9 and 11 WAFS. The fruit K concentration significantly increased 12 WAFS and remained constant until 14 WAFS (Fig. 4-3G).

Calcium. Calcium concentrations in the leaves generally increased as the fruit developed (Fig.4-4A-G). At the end of the 1994 sampling period, the leaf Ca concentration in 'Irwin' was 33% higher than the concentration observed on the first sampling date (Fig. 4-4A). In 1994, 'Irwin' leaves had lower Ca concentrations than 'Tommy Atkins' and 'Van Dyke' leaves throughout fruit ontogeny. Unlike observations made of the leaf Ca concentrations, the fruit Ca concentrations in 'Irwin' continuously

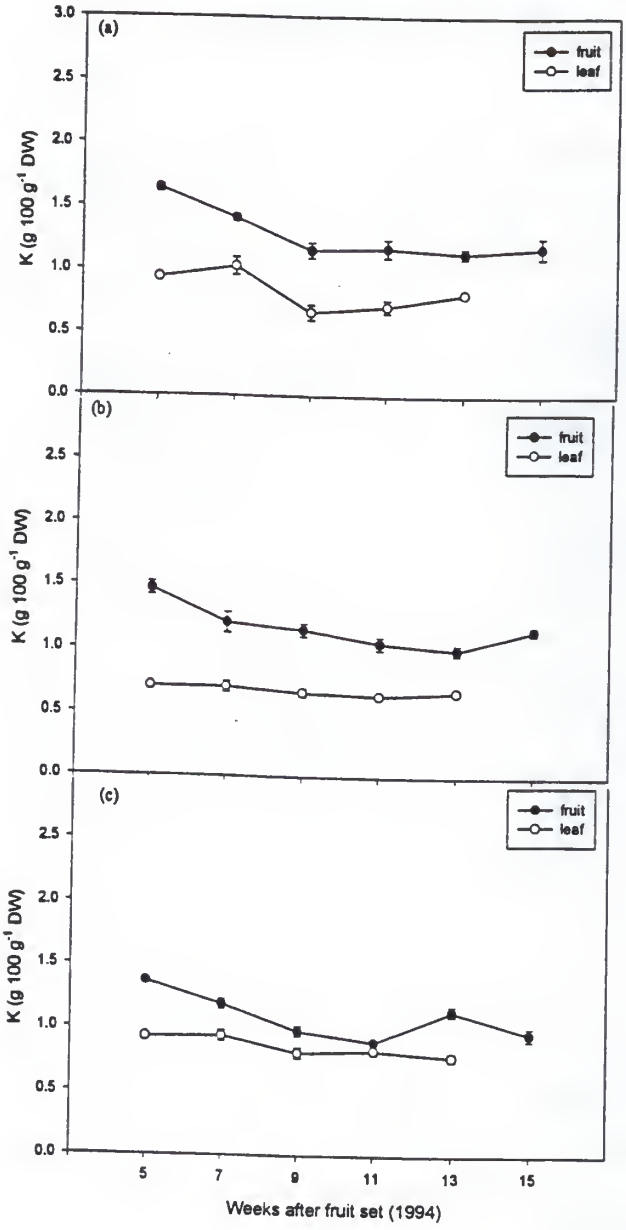
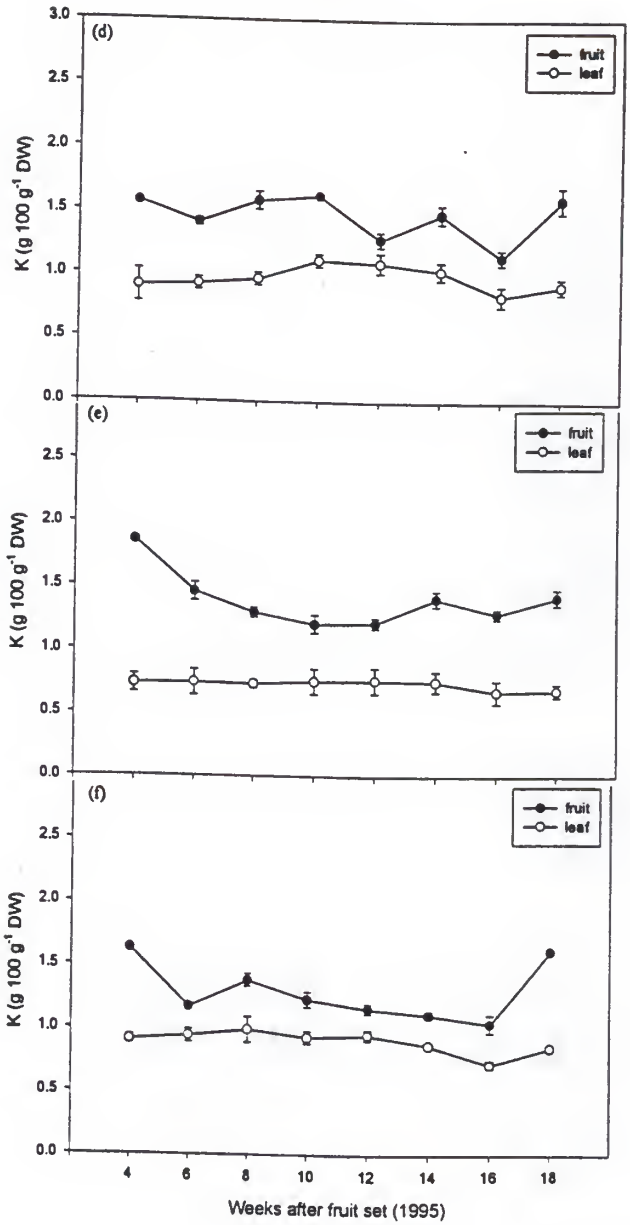
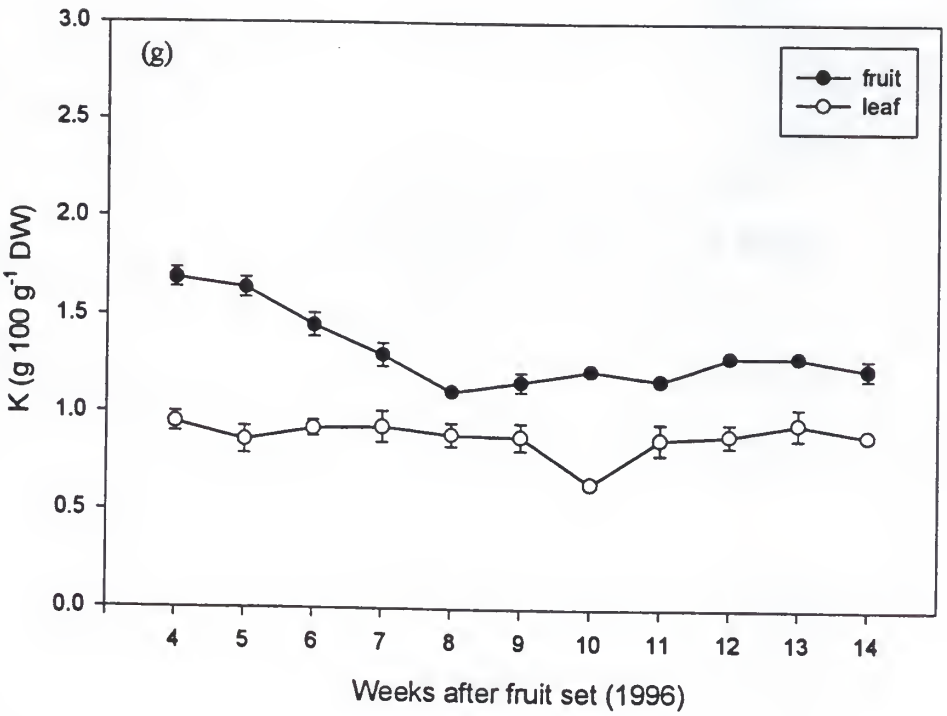


Fig. 4-3. Fluctuations in the K concentrations in 1994 in leaves and fruit of 'Irwin' (a), 'Tommy Atkins' (b), and 'Van Dyke' (c), in 1995 in leaves and fruit of 'Irwin' (d), 'Tommy Atkins' (e), and 'Van Dyke' (f), and in 1996 in leaves and fruit of 'Tommy Atkins' (g), during fruit ontogeny. Values represent means of 25 fruit or 25 mature leaves. Vertical bars represent ± 1 standard error. Absence of error bars indicates that the standard error was smaller than the symbol for the mean.


(d)
(e)
(f)
fruit
leaf
K (g 100 g-1 DW)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
4
6
8
10
12
14
16
18
Weeks after fruit set (1995)


(g)
fruit
leaf
K (g 100 g-1 DW)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
4
5
6
7
8
9
10
11
12
13
14
Weeks after fruit set (1996)

declined throughout fruit ontogeny. When the fruit were ripe, the Ca concentration in 'Irwin' was 48.6% lower than the concentration observed on the first sampling date (Fig. 4-4A). For 'Tommy Atkins', leaf Ca concentration also increased during the fruit development period and the final leaf Ca concentration was 23% higher than the concentration observed on the first sampling date. However, the Ca concentrations in 'Tommy Atkins' fruit declined with time and the fruit Ca concentration on the last sampling date was 54.8% the concentrations observed on the first sampling date (Fig. 4-4B). In 'Van Dyke' trees, leaf Ca concentration showed a continuous increase throughout fruit ontogeny and the final leaf Ca concentration was 41% higher than the Ca concentration observed on the first sampling date. The fruit Ca concentration in 'Van Dyke' declined with time and, at 15 WAFS, was 40.7% of the concentration observed 5 WAFS (Fig.4-4C).

In 1995, the leaf Ca concentrations in 'Irwin' exhibited some fluctuations during fruit development. Six WAFS, the leaf Ca concentration was 75% the concentration observed 4 WAFS but, 8 WAFS, the leaf Ca concentration was 35.7% higher than the concentration observed in the leaves at 6 WAFS. Ten WAFS, the leaf Ca concentration significantly declined to 91.5% of the concentration recorded 8 WAFS, and remained constant between 10 to 14 WAFS. The leaf Ca concentration significantly increased again at 16 WAFS and reached a maximum at 18 WAFS (20% higher than the concentrations observed 4 WAFS) (Fig. 4-4D). The fruit Ca concentration was highest in 'Irwin' at 4 and 6 WAFS, declining 35.2% at 8 WAFS. There were no significant changes in the fruit Ca concentration between 8 and 18 WAFS (Fig.4-4D). In 1995, the leaf Ca concentration in 'Tommy Atkins' also showed an increasing tendency throughout fruit ontogeny. Leaf Ca concentrations were not significantly different at 4 and 6 WAFS. Eight WAFS, the leaf Ca concentration increased 19% the concentration observed 4 WAFS. That increase was followed by a 9.9% decline at 10 WAFS. The leaf Ca concentration increased again 20.6% at 12 WAFS, and remained significantly

constant until the fruit ripened. The final leaf Ca concentration observed 18 WAFS was 38.3% higher than the concentrations observed 4 WAFS (Fig. 4-4E). The fruit Ca concentrations rapidly declined between 4 and 8 WAFS. Then, no significant changes in the fruit Ca concentrations were observed until the fruit ripened (Fig. 4-4E). In 'Van Dyke' trees, the leaf Ca concentrations were lowest at 6 WAFS and highest at 18 WAFS. Six WAFS, the leaf Ca concentration in 'Van Dyke' trees represented 78.4% of the concentration observed 4 WAFS. The leaf Ca concentration increased continuously from 8 to 14 WAFS, at which time the leaf Ca concentration was 26.9% higher than the concentration observed 4 WAFS. Sixteen WAFS, the leaf Ca concentration declined to 14.5% of the concentrations observed at 14 WAFS, but increased to that level again at 18 WAFS (Fig. 4-4F). In 'Van Dyke' fruit, the Ca concentration slightly decreased with time and reached a minimum 16 WAFS. There were no differences in fruit Ca concentrations in 'Van Dyke' fruit between 4 and 8 WAFS. However, the Ca concentrations observed between 4 and 8 WAFS were significantly higher than the concentrations observed between 10 and 18 WAFS.

In 1996, little fluctuation was observed in the leaf Ca concentration of 'Tommy Atkins' trees. In general, leaf Ca concentration continuously increased throughout fruit ontogeny, and was lowest at 4 WAFS and highest at 13 WAFS. Between 6 and 14 WAFS, the leaf Ca concentrations were significantly higher than the concentration observed 4 WAFS. At 11 and 12 WAFS, the leaf Ca concentrations were significantly lower than the concentrations observed at 10 and 13 WAFS. At the end of the sampling period, the final foliar Ca concentration in the 'Tommy Atkins' trees was 36% higher than the concentration observed on the first sampling date (Fig. 4-4G). No significant changes occurred in the Ca concentrations of the 'Tommy Atkins' fruit between 4 and 8 WAFS. Nine WAFS, the fruit Ca concentration was lower than the concentration observed 4 WAFS. The fruit Ca concentrations slightly decreased with time from 9 to

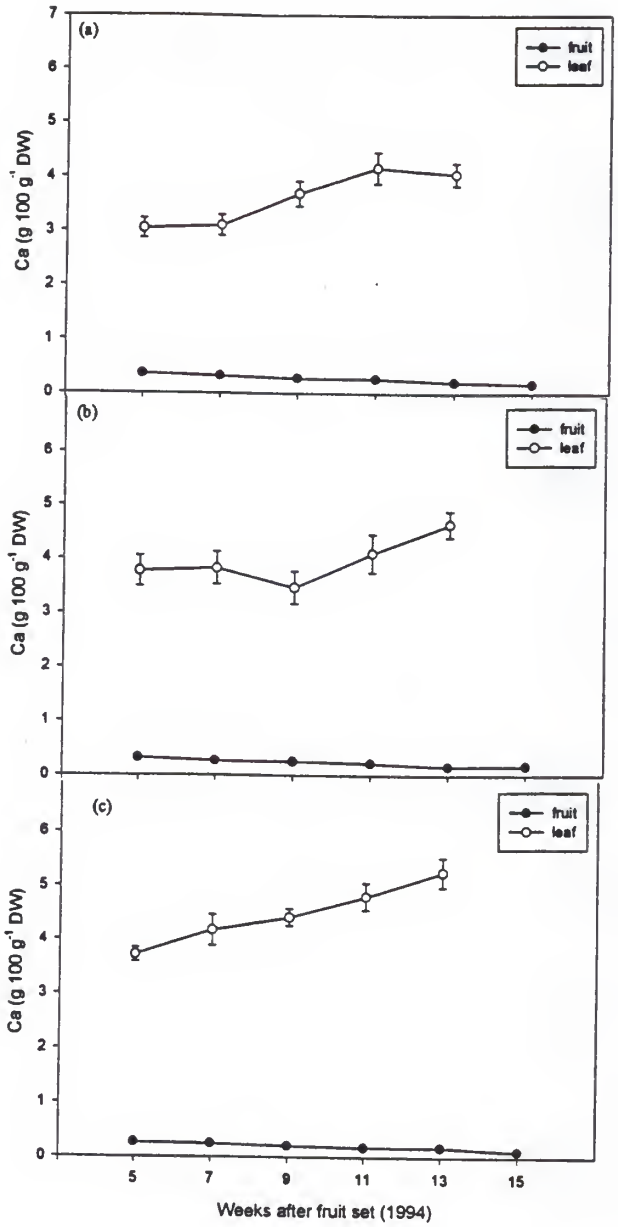
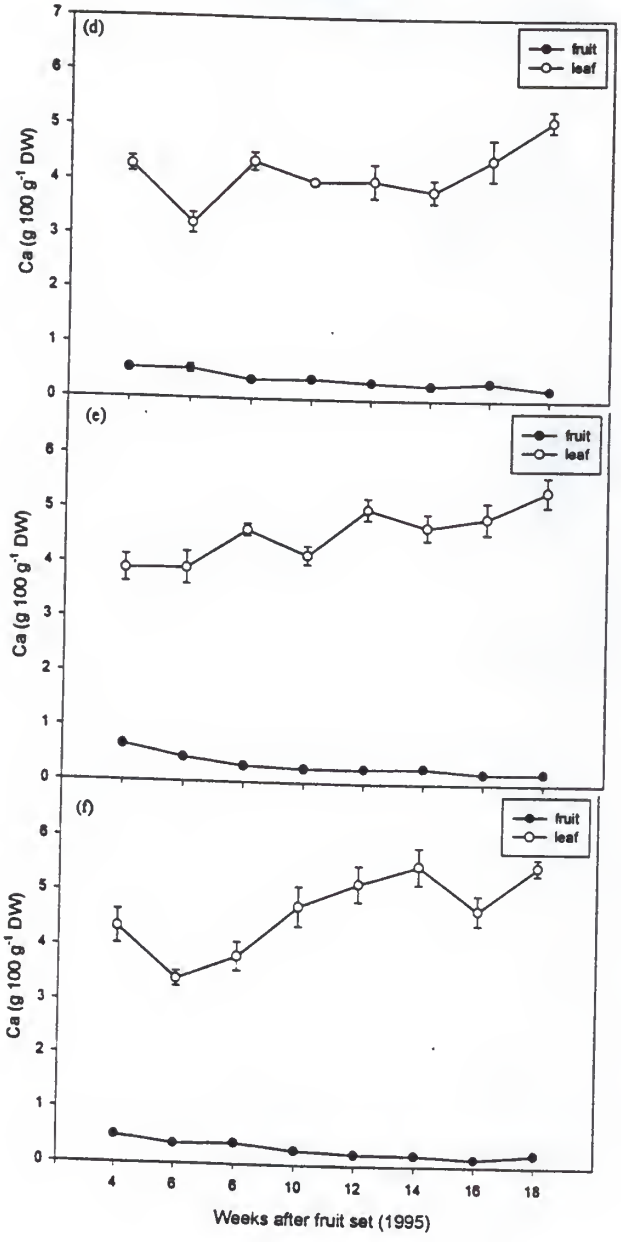
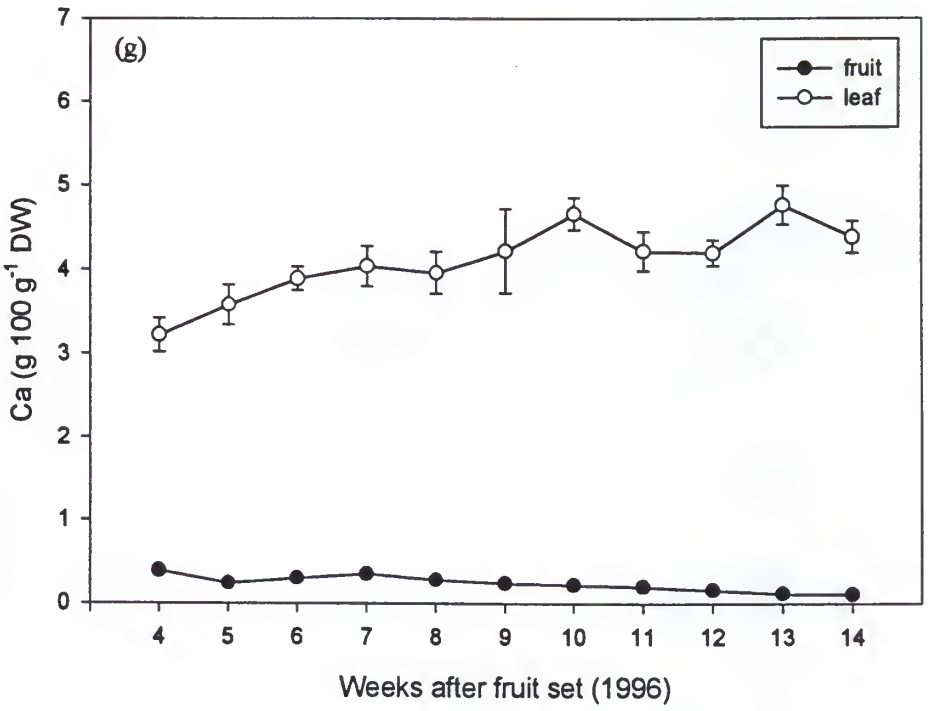


Fig. 4-4. Fluctuations in the Ca concentrations in 1994 in leaves and fruit of 'Irwin' (a), 'Tommy Atkins' (b), and 'Van Dyke' (c), in 1995 in leaves and fruit of 'Irwin'(d), 'Tommy Atkins' (e), and 'Van Dyke' (f), and in 1996 in leaves and fruit of 'Tommy Atkins' (g), during fruit ontogeny. Values represent means of 25 fruit or 25 mature leaves. Vertical bars represent ± 1 standard error. Absence of error bars indicates that the standard error was smaller than the symbol for the mean.


(d)
(e)
(f)
fruit
leaf
Ca (g 100 g-1 DW)
0
1
2
3
4
5
6
7
4
6
8
10
12
14
16
18
Weeks after fruit set (1995)


(g)
fruit
leaf
Ca (g 100 g-1 DW)
0
1
2
3
4
5
6
7
4
5
6
7
8
9
10
11
12
13
14
Weeks after fruit set (1996)

14 WAFS, but no differences in the fruit Ca concentrations were found among sampling dates within that period.

Magnesium. Patterns of Mg accumulation in the leaves differed among cultivars. In 1994, foliar Mg concentration in 'Irwin' increased as the fruit developed, and leaf Mg concentration was lowest 5 WAFS and highest when the fruit were ripe. At the end of the sampling period, the Mg concentration in the leaves of 'Irwin' was 6.9% higher than the concentration observed at 5 WAFS. There were no differences in leaf Mg concentrations between 5 and 7 WAFS. The leaf Mg concentration significantly increased 9 WAFS and remained unchanged until the fruit were ripe (Fig. 4-5A). The fruit Mg concentrations declined with time in 'Irwin'. A rapid decline was observed at 7 and 9 WAFS. Then the fruit Mg concentrations stabilized until the fruit were ripe, at which time the Mg concentration was 61.5% the concentration observed at the first sampling date (Fig. 4-5A).

Unlike observations made for 'Irwin', leaf Mg concentration decreased as the fruit developed in 'Tommy Atkins' and reached the minimum two weeks before the fruit were ripe. Seven WAFS, the foliar Mg concentration was not different from the concentration observed 5 WAFS, but that concentration was higher than the concentrations observed at 9, 11, and 13 WAFS. At 5 and 7 WAFS, there were no significant fluctuation in the Mg concentration in 'Tommy Atkins' fruit. The Mg concentration declined 9 WAFS, remained stable at 11 WAFS, and declined again 13 WAFS to finally increase, 15 WAFS, to the concentration observed at 9 and 11 WAFS (Fig. 4-5B).

There were no significant differences in foliar Mg concentrations between 5 and 7 WAFS in 'Van Dyke'. Nine WAFS, the leaf Mg concentration in 'Van Dyke' significantly declined and remained fairly constant thereafter until 15 WAFS. At the end of the sampling period, the foliar Mg concentration represented 83% of the concentration observed at the first sampling date. The fruit Mg concentration in 'Van

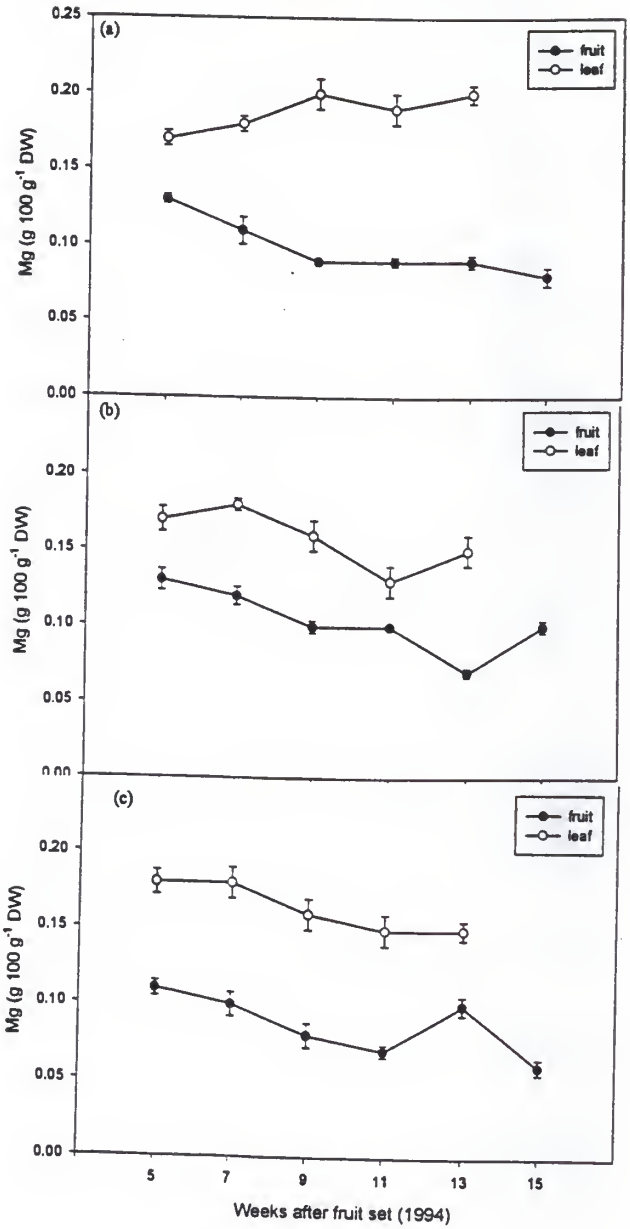


Fig. 4-5. Fluctuations in the Mg concentrations in 1994 in leaves and fruit of 'Irwin' (a), 'Tommy Atkins' (b), and 'Van Dyke' (c), in 1995 in leaves and fruit of 'Irwin' (d), 'Tommy Atkins' (e), and 'Van Dyke' (f), and in 1996 in leaves and fruit of 'Tommy Atkins' (g), during fruit ontogeny. Values represent means of 25 fruit or 25 mature leaves. Vertical bars represent ± 1 standard error. Absence of error bars indicates that the standard error was smaller than the symbol for the mean.

Dyke' declined to 36.4% from 5 to 11 WAFS. That decline in the fruit Mg concentration was followed by a significant increase 13 WAFS, before declining again to a minimum 15 WAFS (Fig. 4-5C). When the fruit were ripe in 1994, no differences in fruit Mg concentrations were observed among the cultivars.

In 1995, the leaf Mg concentrations generally declined throughout fruit ontogeny in all three cultivars. In 'Irwin', Mg concentration in the leaves reached the minimum 12 WAFS. No significant fluctuations occurred in the Mg concentration in 'Irwin' leaves between 4 and 8 WAFS. The leaf Mg concentration significantly decreased 10 and 12 WAFS, remained stable until 16 WAFS before increasing significantly 18 WAFS. The final Mg concentration in 'Irwin' leaves was significantly lower than the concentration observed in the leaves 4 WAFS (Fig. 4-5D). The Mg concentration of 'Irwin' fruit generally declined throughout fruit ontogeny. Eight WAFS, the fruit Mg concentration was 75% of the concentration observed 4 WAFS. No significant changes occurred in the fruit Mg concentration between 8 and 14 WAFS. The Mg concentration in 'Irwin' reached the minimum 16 WAFS. When the fruit were ripe, the Mg concentration in 'Irwin' fruit represented 18.8% of the concentration observed 4 WAFS, but were significantly higher than the concentration observed at 12, 14, and 16 WAFS (Fig. 4-5D).

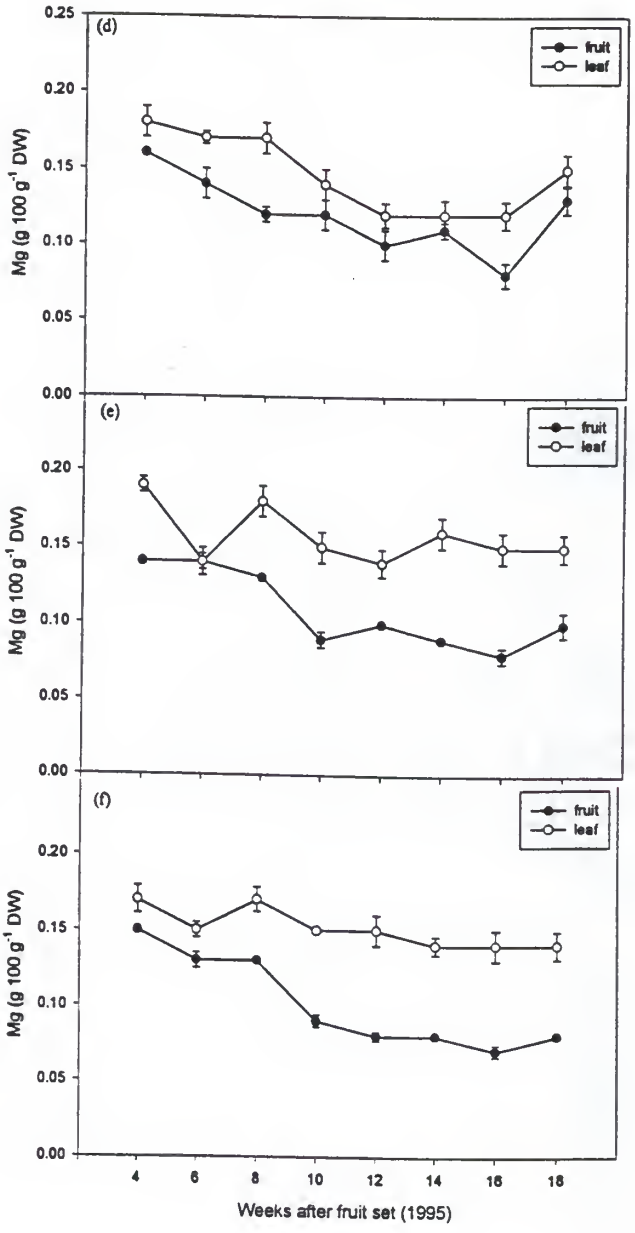
In 'Tommy Atkins', a significant decline occurred in the leaf Mg concentration 6 WAFS. At that sampling date, the leaf Mg concentration was 26.3% of the concentration observed 4 WAFS. The leaf Mg concentration significantly increased 8 WAFS, and declined 10 and 12 WAFS. No significant fluctuation occurred in the Mg concentrations of 'Tommy Atkins' leaves from 10 to 18 WAFS (Fig. 4-5E). The Mg concentration in 'Tommy Atkins' fruit was highest at 4 WAFS and lowest at 16 WAFS. From 4 to 6 WAFS, no changes occurred in the Mg concentration of 'Tommy Atkins' fruit. The Mg concentration in the fruit rapidly declined from 8 to 10 WAFS, and increased again 12 WAFS. The fruit Mg concentration declined again from 14 to

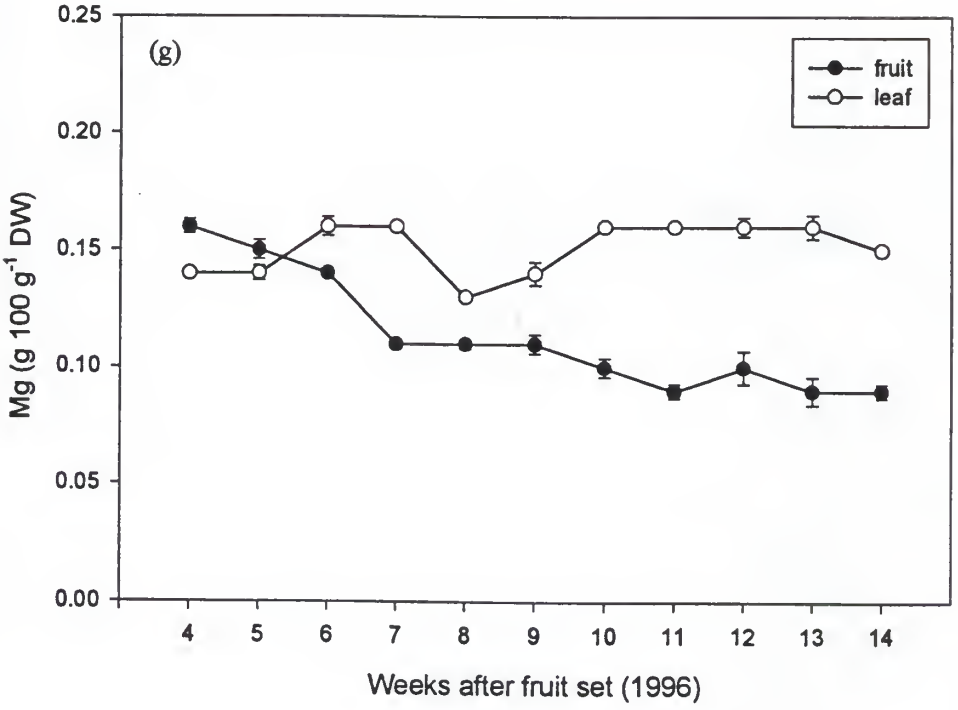
16 WAFS, to finally increase 18 WAFS. When the fruit were ripe, the Mg concentrations in the 'Tommy Atkins' fruit represented 71.4% of the concentrations observed at 4 WAFS (Fig. 4-5E).

The foliar Mg concentration in 'Van Dyke' was highest 4 and 8 WAFS and lowest during the remaining of the fruit development period. From 10 to 18 WAFS, the leaf Mg concentration in 'Van Dyke' remained equal to the concentration observed 6 WAFS (Fig. 4-5F). In the fruit of 'Van Dyke', the Mg concentrations decreased with time and reached a minimum 16 WAFS. No differences in the fruit Mg concentration were found between 6 and 8 WAFS, or between 12 and 14 WAFS. The Mg concentration in the ripe fruit represented 53.3% of the concentration observed 4 WAFS and were significantly higher than the concentration observed 16 WAFS (Fig. 4-5F).

In 1996, the leaf Mg concentrations were lower than the concentrations observed of 'Tommy Atkins' fruit 4 and 5 WAFS. The leaf Mg concentration increased at 6 and 7 WAFS, whereas the fruit Mg concentration declined during the same period. No significant fluctuations occurred in the fruit Mg concentration from 7 to 9 WAFS, but a significant decline was recorded in the leaf Mg concentration 8 WAFS followed by a slight increase 9 WAFS. Ten WAFS, the leaf Mg concentration increased again and remained constant until 16 WAFS. The fruit Mg concentration reached the lowest point at 11 WAFS after decreasing slowly from the concentrations observed 9 and 10 WAFS (Fig. 4-5G).

Zinc. Concentrations of Zn in the leaves were consistently higher than they were in the fruit during the 3 years of the study (Fig. 4-6A-G). In 1994, the Zn concentrations in the leaves of 'Irwin' significantly decreased when the fruit were 6 weeks old (Fig. 4-6A), and remained unchanged until the fruit were 12 weeks old. Despite a noticeable rise when the fruit were ripe, the leaf Zn concentration in 'Irwin' was, at the end of the 1994 sampling period, lower than the concentration observed on


(d)
(e)
(f)
fruit
leaf
Mg (g 100 g-1 DW)
0.25
0.20
0.15
0.10
0.05
0.00
4
6
8
10
12
14
16
18
Weeks after fruit set (1995)


(g)
fruit
leaf
0.25
0.20
0.15
0.10
0.05
0.00
Mg (g 100 g-1 DW)
4
5
6
7
8
9
10
11
12
13
14
Weeks after fruit set (1996)

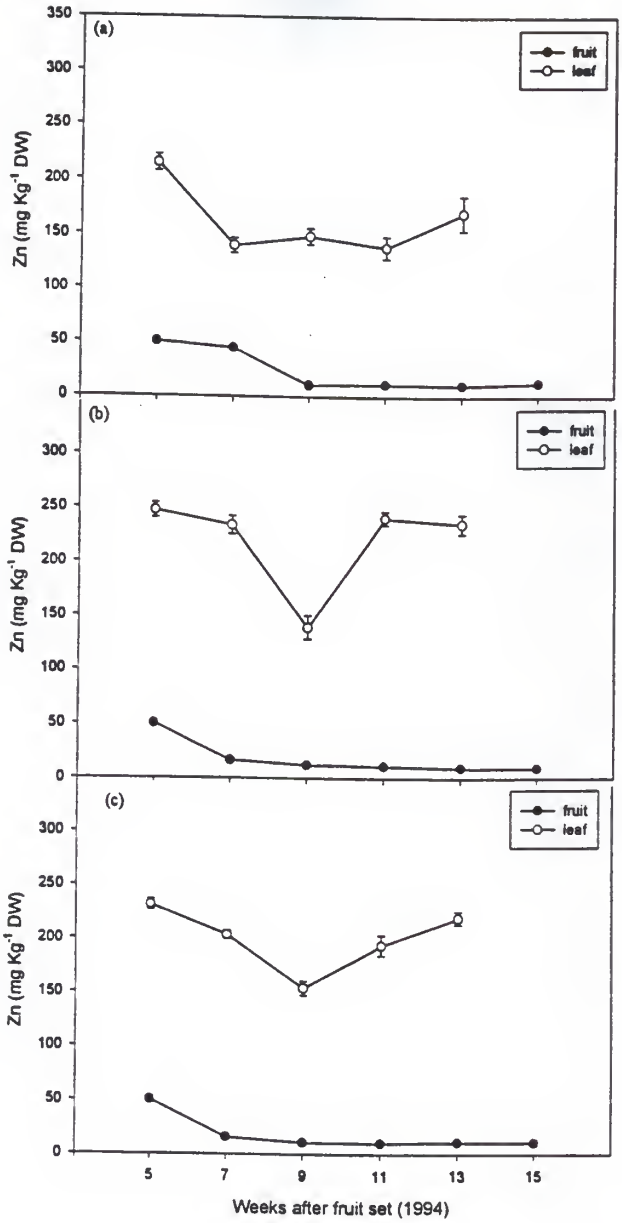
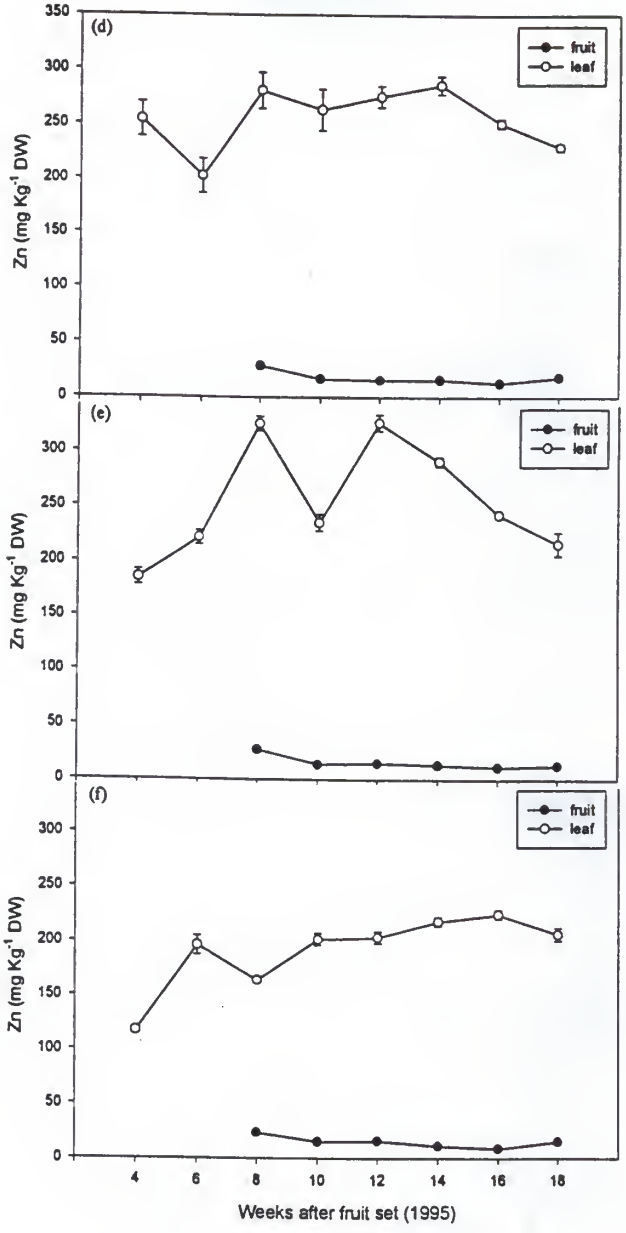


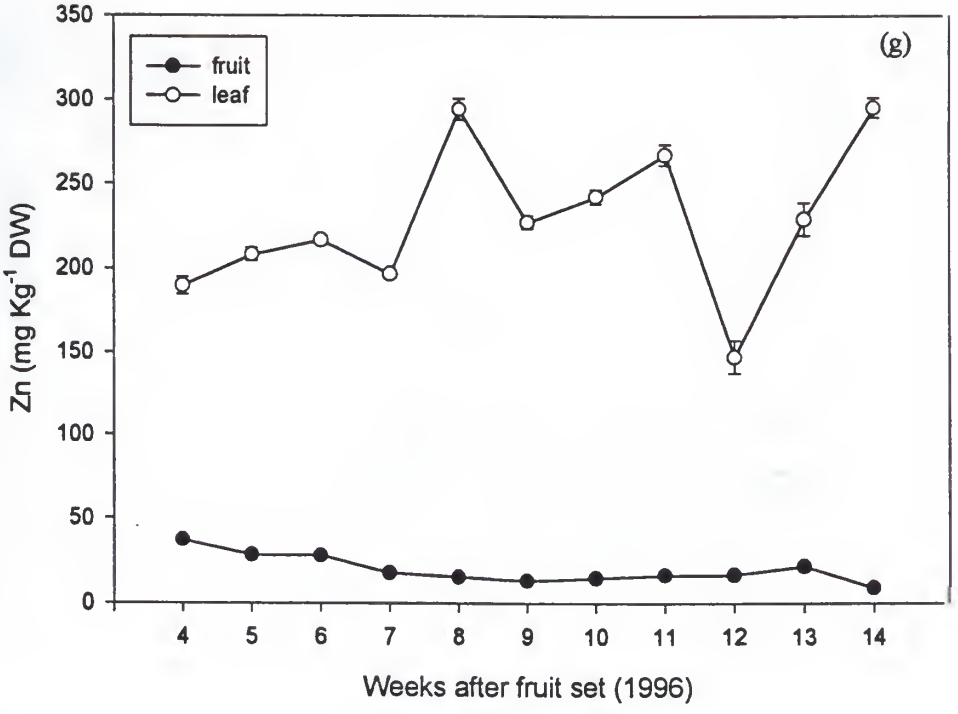
Fig. 4-6. Fluctuations in the Zn concentrations in 1994 in leaves and fruit of 'Irwin' (a), 'Tommy Atkins' (b), and 'Van Dyke' (c), in 1995 in leaves and fruit of 'Irwin' (d), 'Tommy Atkins' (e), and 'Van Dyke' (f), and in 1996 in leaves and fruit of 'Tommy Atkins' (g), during fruit ontogeny. Values represent means of 25 fruit or 25 mature leaves. Vertical bars represent ± 1 standard error. Absence of error bars indicates that the standard error was smaller than the symbol for the mean.

the first sampling date (Fig. 4-6A). Foliar Zn concentrations were higher in 'Tommy Atkins' and 'Van Dyke' (Fig. 4-6B-C), than in 'Irwin' when the fruit were 6 weeks old. The leaf Zn concentration was lowest when the fruit were 8 weeks old, but increased again to the concentrations observed when the fruit were 6 weeks old.

In 1995, the Zn concentration in the leaves exhibited a pattern different from the observations made in 1994. For example, the leaf Zn concentration in 'Irwin' (Fig. 4-6D) was lowest when the fruit were 6 weeks old, while the lowest leaf Zn concentrations in 'Tommy Atkins' and 'Van Dyke' were observed when the fruit were 4 weeks old. Data from the 1995 study (Fig 4-6D-F) showed that the leaf Zn concentration was lower at 15 WAFS than at 4 WAFS in 'Irwin', whereas the leaf Zn concentration at that sampling date in 'Tommy Atkins' and 'Van Dyke' was significantly higher than that observed 4 WAFS. During most of the 1995 sampling period, 'Van Dyke' leaves had lower Zn concentrations than 'Tommy Atkins' or 'Irwin' leaves. No differences in leaf Zn concentrations existed among the cultivars on the last sampling date. In 1996, foliar Zn concentrations in 'Tommy Atkins' showed frequent fluctuations and reached a minimum 2 weeks before the fruit were ripe (Fig. 4-6G). Fruit Zn concentrations remained fairly constant in all the cultivars throughout fruit development (Fig. 4-6A-G). Concentrations of Zn in the fruit decreased early during fruit ontogeny, particularly when the fruit were between 6 and 8 weeks old.

Copper. Concentrations of Cu were higher in the leaves than in the fruit of all 3 cultivars. In 1994 and 1995, leaf Cu concentrations changed little until 2 to 4 weeks before the fruit were ripe (Fig. 4-7A-F). Foliar Cu concentrations did not significantly differ among the three cultivars during 1994 (Fig. 4-7A-C). In 1995, 'Irwin' and 'Tommy Atkins' leaves contained higher Cu concentrations than 'Van Dyke' leaves, except when fruit were 12 and 18 weeks old. Copper concentrations in the fruit changed little throughout fruit development (Fig. 4-7A-G).


(d)
(e)
(f)
fruit
leaf
Zn (mg Kg-1 DW)
0
50
100
150
200
250
300
350
4
6
8
10
12
14
16
18
Weeks after fruit set (1995)


(g)
fruit
leaf
Zn (mg Kg-1 DW)
350
300
250
200
150
100
50
0
4
5
6
7
8
9
10
11
12
13
14
Weeks after fruit set (1996)

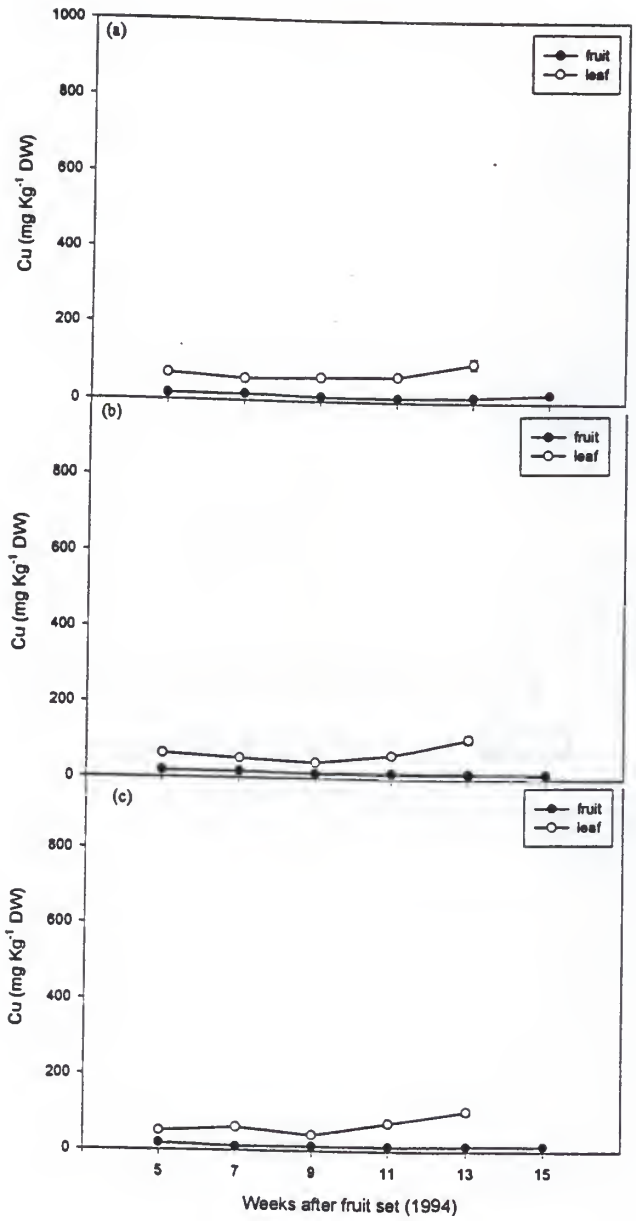
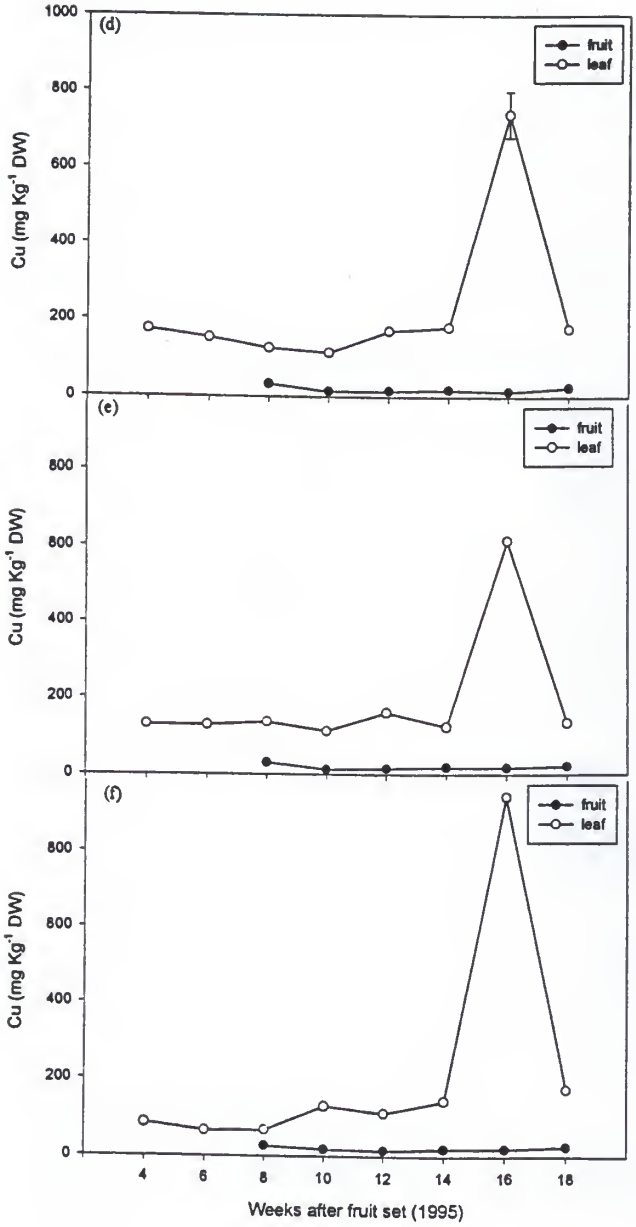
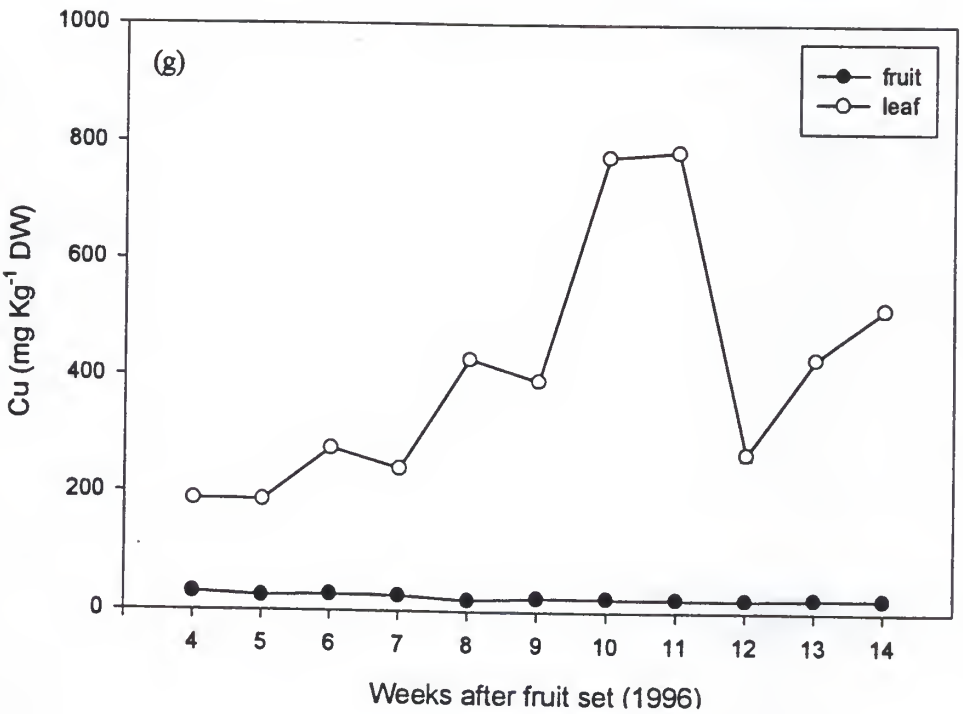


Fig. 4-7. Fluctuations in the Cu concentrations in 1994 in leaves and fruit of 'Irwin' (a), 'Tommy Atkins' (b), and 'Van Dyke' (c), in 1995 in leaves and fruit of 'Irwin' (d), 'Tommy Atkins' (e), and 'Van Dyke' (f), and in 1996 in leaves and fruit of 'Tommy Atkins' (g), during fruit ontogeny. Values represent means of 25 fruit or 25 mature leaves. Vertical bars represent ± 1 standard error. Absence of error bars indicates that the standard error was smaller than the symbol for the mean.


(d)
(e)
(f)
fruit
leaf
Cu (mg Kg-1 DW)
0
200
400
600
800
1000
4
6
8
10
12
14
16
18
Weeks after fruit set (1995)


(g)
fruit
leaf
1000
800
600
400
200
0
Cu (mg Kg-1 DW)
4
5
6
7
8
9
10
11
12
13
14
Weeks after fruit set (1996)

Manganese. In 1994, there was a decline in foliar Mn concentration when the fruit were approximately 9 to 11 weeks old. The highest concentrations of leaf Mn were reached 2 weeks before the fruit were ripe (Fig. 4-8A-C). In 1995 and 1996, the fluctuations in leaf Mn concentrations differed among the three cultivars (Fig. 4-8D-G). 'Irwin' and 'Tommy Atkins' leaves contained higher Mn concentrations than 'Van Dyke' leaves. Manganese concentrations were slightly higher in young fruit than in ripe fruit(Fig. 4-8A-G). Fruit Mn concentrations slightly decreased during the first 8 to 12 weeks of fruit development, and then remained stable until the fruit were ripe. Prior to ripening, 'Irwin' fruit contained higher concentrations of Mn than 'Tommy Atkins' or 'Van Dyke' fruit but there were no differences in Mn concentrations among the cultivars in ripe fruit.

Iron. Foliar concentrations of Fe generally remained stable between weeks 5 and 11 in 1994 (Fig. 4-9A-C) and weeks 4 and 10 in 1995 (Fig. 4-9D-F), thereafter increasing sharply to a peak (Fig 4-9A-C) until 2 weeks before the fruit were ripe in 1995. In 1996, concentrations of Fe in the leaves of 'Tommy Atkins' changed little between 4 and 7 WAFS, then increased to a peak at week 10. Leaf Fe concentrations then declined to a minimum at week 12 and subsequently increased until the fruit were ripe at 14 WAFS (Fig. 4-9G). Leaf Fe concentrations were generally not different between 'Irwin' and 'Van Dyke'. In 1995, Fe concentration were higher in ripe 'Irwin' fruit than in ripe 'Tommy Atkins' or 'Van Dyke' fruit. In 1994 and 1995, five-week-old 'Van Dyke' fruit had higher Fe concentrations than 'Irwin' or 'Tommy Atkins' fruit of the same age. In 1995 and 1996, the youngest fruit had significantly higher Fe concentrations than ripe fruit (Fig. 4-9A-G). Fruit Fe concentrations reached their lowest levels about midway through fruit development, approximately during the seventh to tenth WAFS.

Boron. In 1994, leaf B concentrations reached a maximum at 9 WAFS in 'Irwin', at 7 WAFS in 'Tommy Atkins', and at 11 WAFS in 'Van Dyke'. In 'Irwin'

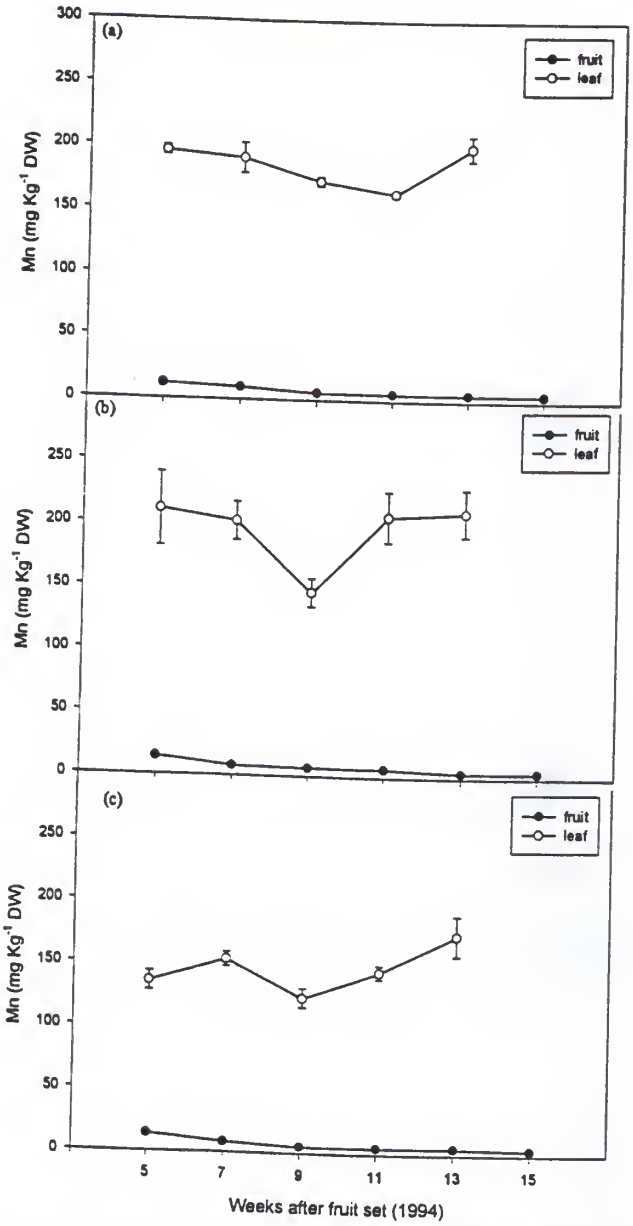
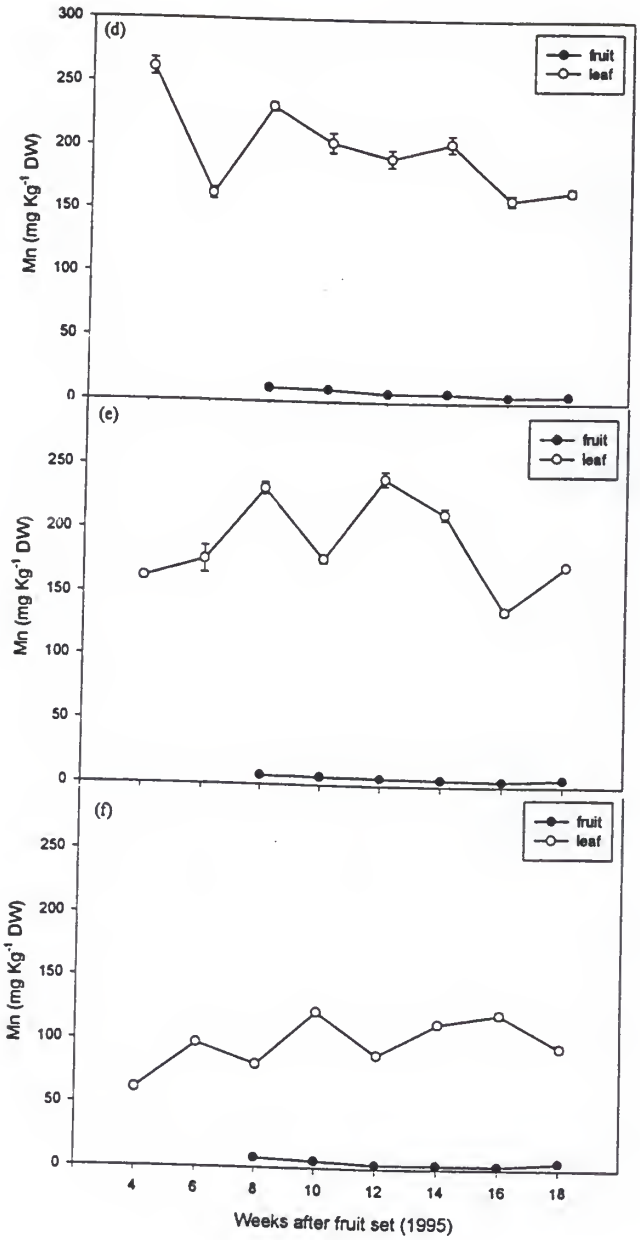
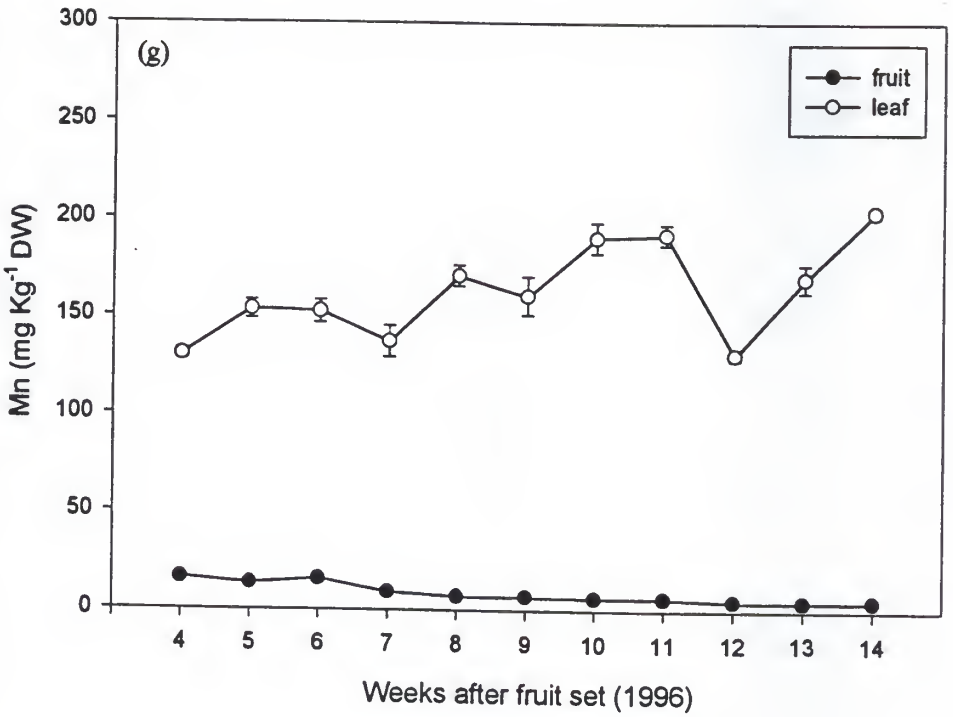


Fig. 4-8. Fluctuations in the Mn concentrations in 1994 in leaves and fruit of 'Irwin' (a), 'Tommy Atkins' (b), and 'Van Dyke' (c), in 1995 in leaves and fruit of 'Irwin' (d), 'Tommy Atkins' (e) and 'Van Dyke' (f), and in 1996 in leaves and fruit of 'Tommy Atkins' (g), during fruit ontogeny. Values represent means of 25 fruit or 25 mature leaves. Vertical bars represent ± 1 standard error. Absence of error bars indicates that the standard error was smaller than the symbol for the mean.


(d)
(e)
(f)
fruit
leaf
Mn (mg Kg-1 DW)
300
250
200
150
100
50
0
4
6
8
10
12
14
16
18
Weeks after fruit set (1995)


(g)
fruit
leaf
Mn (mg Kg-1 DW)
300
250
200
150
100
50
0
4
5
6
7
8
9
10
11
12
13
14
Weeks after fruit set (1996)

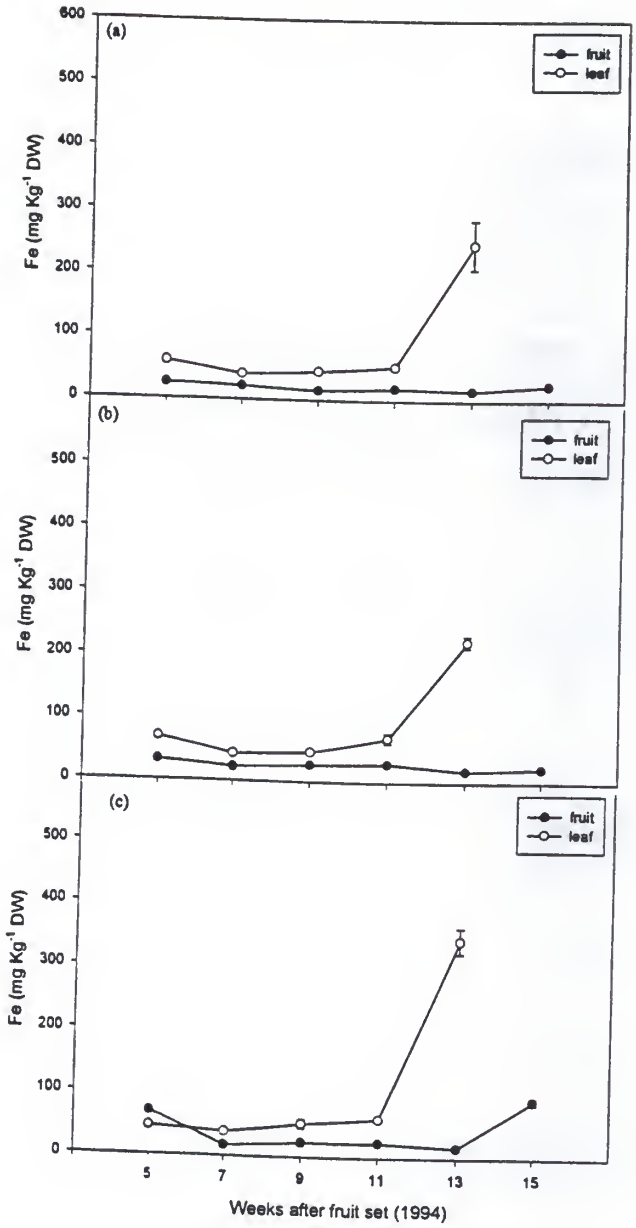
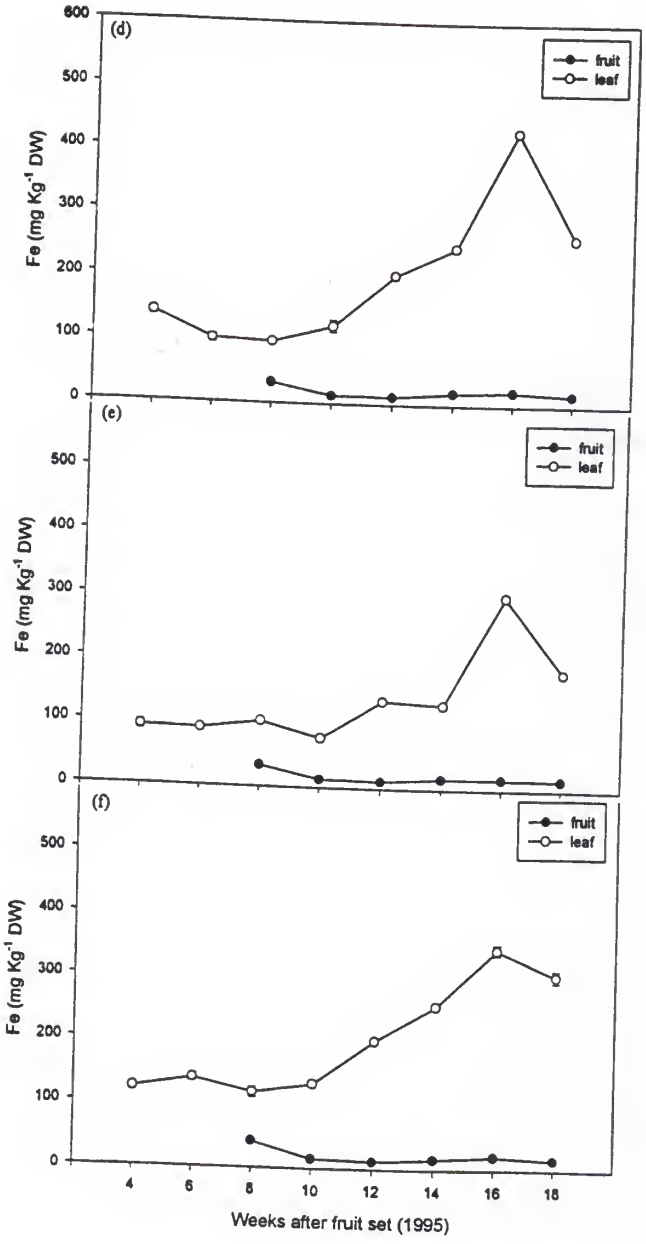
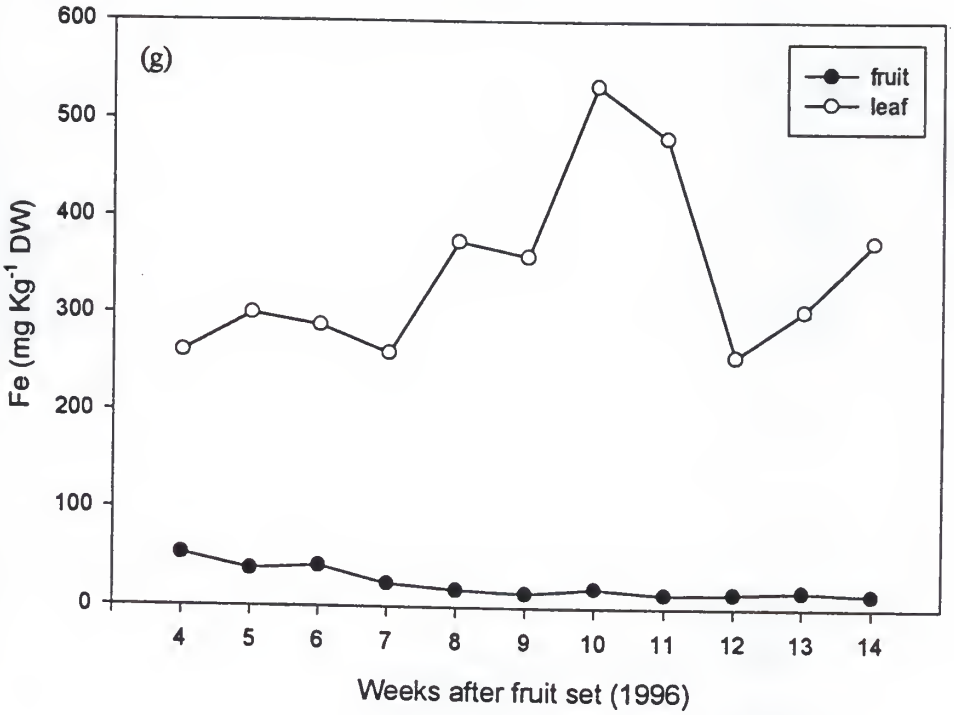


Fig. 4-9. Fluctuations in the Fe concentrations in 1994 in leaves and fruit of 'Irwin' (a), 'Tommy Atkins' (b), and 'Van Dyke' (c), in 1995 in leaves and fruit of 'Irwin' (d), 'Tommy Atkins' (e), and 'Van Dyke' (f), and in 1996 in leaves and fruit of 'Tommy Atkins' (g), during fruit ontogeny. Values represent means of 25 fruit or 25 mature leaves. Vertical bars represent ± 1 standard error. Absence of error bars indicates that the standard error was smaller than the symbol for the mean.


(d)
(e)
(f)
fruit
leaf
Fe (mg Kg-1 DW)
0
100
200
300
400
500
600
4
6
8
10
12
14
16
18
Weeks after fruit set (1995)


(g)
fruit
leaf
600
500
400
300
200
100
0
Fe (mg Kg-1 DW)
4
5
6
7
8
9
10
11
12
13
14
Weeks after fruit set (1996)

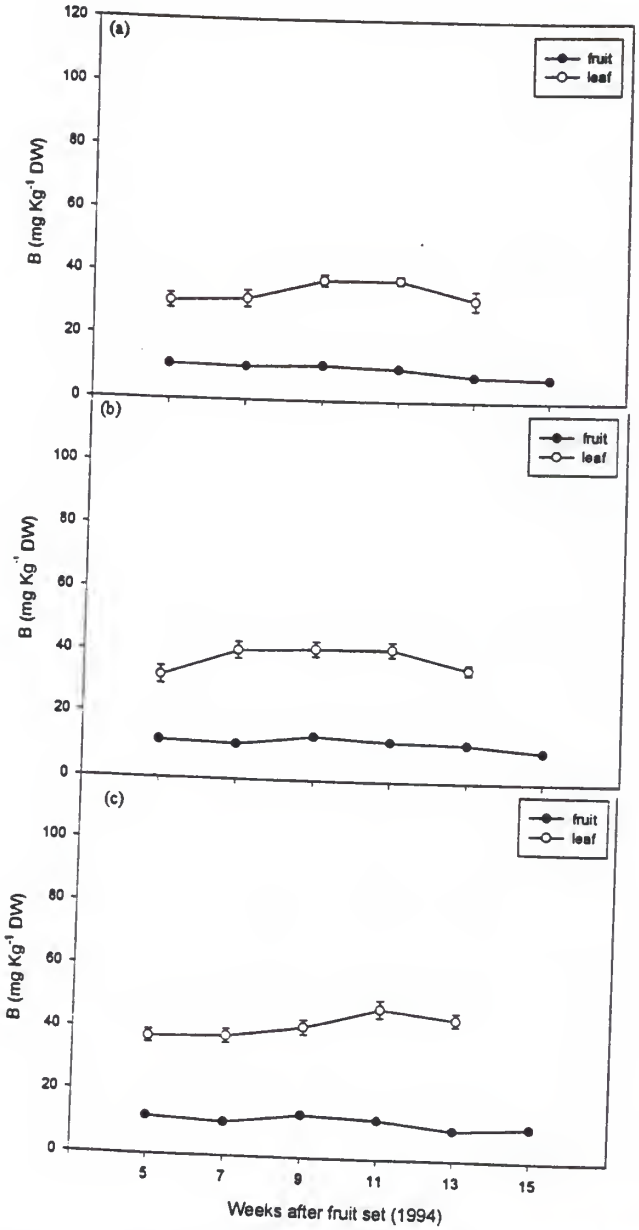
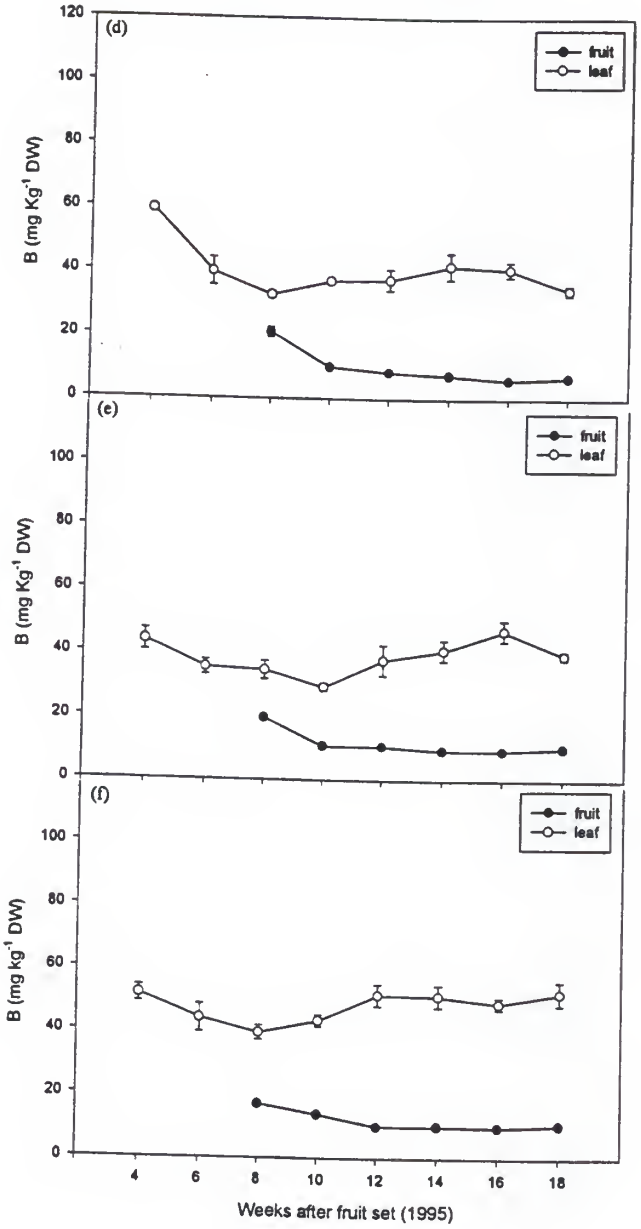
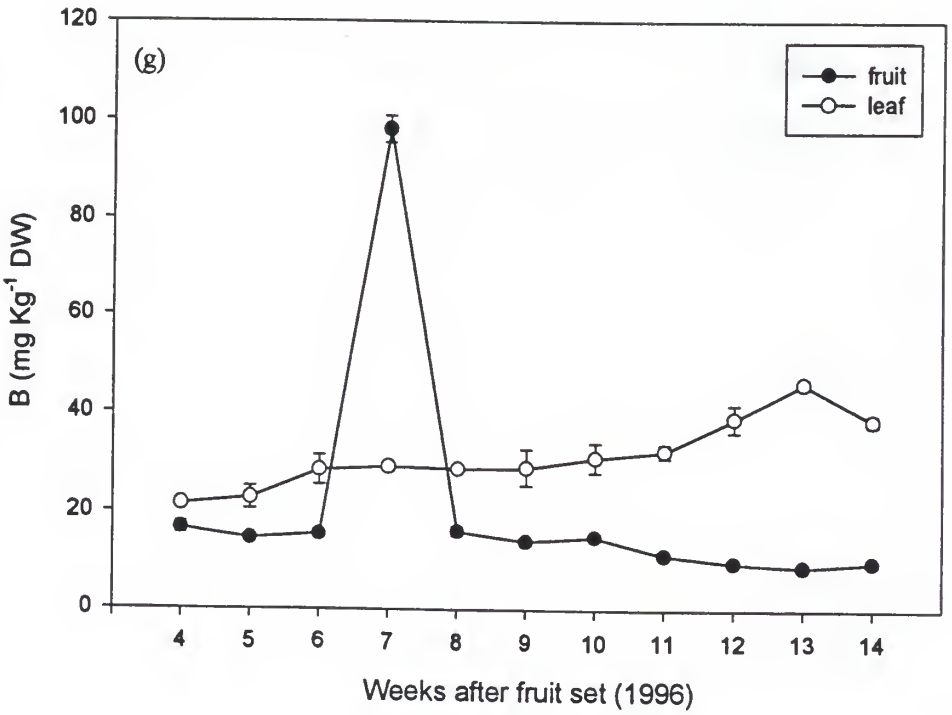


Fig. 4-10. Fluctuations in the B concentrations in 1994 in leaves and fruit of 'Irwin' (a), 'Tommy Atkins' (b), and 'Van Dyke' (c), in 1995 in leaves and fruit of 'Irwin' (d), 'Tommy Atkins' (e), and 'Van Dyke' (f) and in 1996 in leaves and fruit of 'Tommy Atkins' (g), during fruit ontogeny. Values represent means of 25 fruit or 25 mature leaves. Vertical bars represent ± 1 standard error. Absence of error bars indicates that the standard error was smaller than the symbol for the mean.


(d)
fruit
leaf
B (mg Kg-1 DW)
120
100
80
60
40
20
0
(e)
fruit
leaf
B (mg Kg-1 DW)
100
80
60
40
20
0
(f)
fruit
leaf
B (mg kg-1 DW)
100
80
60
40
20
0
4
6
8
10
12
14
16
18
Weeks after fruit set (1995)


(g)
fruit
leaf
B (mg Kg-1 DW)
120
100
80
60
40
20
0
4
5
6
7
8
9
10
11
12
13
14
Weeks after fruit set (1996)

and 'Tommy Atkins, there were no differences in leaf B concentrations between the first and last sampling dates. However on the last sampling date, the B concentration in 'Van Dyke' leaves was higher than the B concentration observed on the first sampling date. In 1994, the fruit B concentration in the three cultivars exhibited no significant change throughout fruit ontogeny (Fig. 4-10A-C). In 1995, the foliar B concentration in 'Irwin' was highest 4 WAFS, and declined to the minimum at 8 WAFS (Fig 4-10D-F). In 'Tommy Atkins', there was little fluctuation in the leaf B concentration throughout fruit ontogeny. The leaf B concentration was lowest at 10 WAFS in 'Tommy Atkins' and at 8 WAFS in 'Van Dyke'. There were no differences in leaf B concentration between the first and the last sampling dates in 'Tommy Atkins'. In 1995, the fruit B concentration was highest at 8 WAFS in all three cultivars, and lowest at 10 WAFS in 'Irwin' and 'Tommy Atkins' fruit. The lowest concentration in fruit B concentration was observed at 12 WAFS in 'Van Dyke'. After the minimum was, the fruit B concentration remained unchanged in all three cultivars until the fruit were ripe (Fig.4-10D-F). In 1996, foliar B concentrations continuously increased throughout fruit ontogeny and B concentration was highest 1 week before the fruit were (Fig. 4-10G). Except for an inexplicable increase observed at 7 WAFS, the concentrations of B in the leaves remained consistently higher than in the fruit, and an inverse relationship was observed between leaf and fruit B concentration until 13 WAFS. In general, the fruit B concentration consistently decreased during fruit development, and was lowest one week before the fruit were ripe (Fig. 4-10G).

Discussion

The results reported in this study indicate that the highest concentrations of N, Ca, and K are found in mango leaves and fruit at the beginning of the fruiting period.

Mineral elements selectively accumulate in specific organs. For example, except for P and K, higher concentrations of mineral elements were found in leaves.

There is limited information in the literature about the mineral status of mango leaves and fruit during fruit ontogeny. Avilán (1971) studied the variations in N, P, K, and Ca in the leaves of 14-year-old' Kent' mango trees in Venezuela throughout a production cycle. Leaves were sampled during a 1-year period and three samples were collected during the fruiting period: at fruit set, at fruit maturity, and at harvest. Avilán found that leaf N levels were 1.04%, 1.05%, 1.12% of the dry weight at fruit set, at fruit maturity, and at harvest, respectively. Leaf P and K concentrations were also higher at harvest than at fruit set. However, leaf Ca decreased from 2.77 % at fruit set to 2.2% at harvest. Young and Koo (1971) investigated the effects of age and position on N, P, K, Ca, and Mg concentrations in 'Irwin' mango leaves. Seven samplings were made in 1 year, two of which were made during the fruiting period. They observed decreasing concentrations of N, P, and K with time, whereas leaf Ca concentrations increased. In that study, no significant fluctuations were observed in Mg concentrations with time. Lakshminarayana (1980) reported the chemical composition of mango fruit from 0 to 16 WAFS. The total fruit N showed a rapid decline from 0 to 6 WAFS and remained fairly constant until 16 WAFS. The fluctuations of the other mineral elements were not reported in that study.

Table 4-1 shows the averages of macronutrients concentrations in the leaves and fruit of the three cultivars throughout this 3-year study. These data indicate that leaves contained higher concentrations of N, Ca, and K, whereas P and K were in higher concentrations in fruit.

On the first sampling date, the order of magnitude of macronutrient concentrations in the leaves was Ca>N>K>Mg>P; in fruit the order was K>N>Ca>P>Mg, and these orders did not change when the fruit were ripe.

Table 4-1. Concentrations of five macronutrients in mango leaves and fruit early in fruit development and when fruit were ripe.

Organ sampled	Stage of fruit devp.	g 100 g^{-1} DW[z]				
		N	P	K	Ca	Mg
Fruit	5-week-old	1.61	0.25	1.62	0.42	0.14
	Ripe	0.60	0.11	1.29	0.16	0.09
Leaf	5-week-old	1.16	0.08	0.89	3.63	0.16
	Ripe	1.14	0.08	0.82	4.80	0.16

[z]: DW= Dry weight.

Leaf N concentrations fluctuated with time and among cultivars. Leaf N concentration increased during fruit development in 'Irwin' and 'Van Dyke' but decreased in 'Tommy Atkins'. Avilán (1971) also reported increasing leaf N concentrations from fruit set to harvest in 'Kent' mangoes in Venezuela. However, Koo and Young (1972) observed decreasing leaf N concentrations with time in 'Irwin' mangoes. These differences could be due to the differences in the sampling intervals, or might also be cultivar-related. For example, Young and Koo (1971) investigated the variations of mineral content of mango leaves in Florida and observed higher leaf N concentrations in 'Kent' compared to 'Tommy Atkins' or 'Keitt' leaves. Leaf samples were collected three times in Avilán's study whereas Young and Koo (1971) collected leaf samples only once during the fruiting period. Consequently, it was not possible for Young and Koo to detect N fluctuations during fruit development. Differences in cultural conditions (e.g. soil fertility level, fertilizer program) might have affected the availability of nutrients to the roots. In the present study, fluctuations observed in leaf N concentrations were in the range of 0.98-1.25g 100 g^{-1} on a dry weight basis. Leaf N concentrations observed from 1994 to 1996 were within the range observed by Avilán (1971) for 'Kent' in Venezuela and Young and Koo (1969) in Florida for 'Kent',

'Haden', 'Parvin', and 'Zill'. They were, however, below the 1.35-2.32% range recommended by de Laroussilhe (1980).

Fruit N concentration rapidly declined 8 WAFS. De Laroussilhe (1980) indicated that the mango fruit enters a rapid growth phase 3 to 4 WAFS, after the cell division and multiplication phase which lasts 21 days. The reductions in fruit N concentrations that were observed appear to be normal and may be a consequence of nutrient dilution due to fruit enlargement. Eight WAFS, N concentration in the fruit was approximately 50% of the concentration observed on the first sampling date. Lakshminarayana (1980) reported a similar reduction in the total N content for 'Alphonso' mango fruit. In the present study, N concentrations in ripe fruit were 41.1%, 38%, and 42.5% of the N concentrations observed on the first sampling date for 'Irwin', 'Tommy Atkins', and 'Van Dyke', respectively.

Phosphorus concentrations remained more constant in mango leaves than in fruit. In general, the fruit in this study contained higher P concentrations than the leaves. The lowest foliar P concentration observed in the leaves was 0.07% while the maximum was 0.10%. Avilán (1971), Koo and Young (1972), and Young and Koo (1969, 1971) determined that the sufficient range for P in mango leaves should be between 0.07% and 0.175% on a dry weight basis. However, de Laroussilhe (1980) determined that the ideal P concentrations for mango leaves was 0.14% to 0.17%. In the fruit, P concentrations fluctuated between 0.07% and 0.37%. Phosphorus concentrations were highest in the youngest fruit. Phosphorus is an integral part of phospholipids of cell membranes and nuclei (Bidwell, 1979). Phosphorus is also a component of nucleic acids and other molecules such as adenosine diphosphate (ADP) and adenosine triphosphate (ATP). Synthesis of phospholipids and nucleic acid is essential for newly formed cells resulting from cell division. Thus, the need for P is high during cell division. Shortly after fruit set, young mango fruit enter the cell division stage, which lasts approximately 3 weeks (de Laroussilhe, 1980). This may

explain the existence of the higher fruit P levels observed at the beginning of the fruiting period compared to lower levels found in mature fruit. Low concentrations of P in the oldest fruit may also be the result of dilution due to cell growth and enlargement following cell division.

Leaf K concentrations ranged from 0.58 to 1.03 g $100g^{-1}$ in this study, whereas fruit K concentrations fluctuated between 0.9 and 2.01 g $100g^{-1}$. Recommendations vary greatly as to the ideal K concentration in mango leaves. De Laroussilhe (1980) suggested that leaf K concentration should be 0.64% on a dry weight basis, whereas Young and Koo (1969) and Cull (1991) recommended 0.3-0.8%. However, Avilán (1971) recommended that leaf K concentration should range from 1.04% to 1.24%. Laborem et al. (1979) investigated the concentrations of mineral elements in 16 mango cultivars, including 'Tommy Atkins' and 'Irwin', in Venezuela. In that study, K concentrations in ripe fruit averaged 2.38% of the dry weight. Differences in K concentrations among studies may be due to environmental conditions, physiological status of the plants at the time of sampling, and cultural practices. Potassium is involved in several plant processes, e.g. photosynthesis, photophosphorylation, respiration, protein and starch synthesis, and stomatal movement (Bidwell, 1979). For these processes to occur, K must be constantly supplied at required levels to the plant. Although K concentrations in leaves fluctuated slightly over time, there was no significant increase when fruit were ripe. Thus, K supply was probably adequately maintained throughout the fruiting period. Potassium translocates readily from older tissues to growing organs (Follett et al., 1981; Hewitt and Smith, 1985). The high K concentrations observed in the youngest fruit may have been due to translocation from leaves. However some dilution may subsequently have occurred, resulting in decreased K concentrations during fruit development.

Foliar Ca concentrations continuously increased during fruit development in this study. In some cases, leaf Ca concentrations on the last sampling date were almost

twice the levels initially observed in the leaves at the first sampling date. Leaf Ca concentrations ranged from 3.05 g 100 g^{-1} to 5.86 g 100 g^{-1}. These values are well above the 0.91% sufficiency level suggested by de Laroussilhe (1980). However, they are similar to the 3.0%-5.0% range reported by Young and Koo (1969) in an alkaline soil in Florida. Young and Koo reported that leaf Ca concentrations ranged from 2.0%-3.5% in acid soils. Calcium is relatively immobile in plants (Clarkson, 1984; Demarty et al., 1984; Hewitt and Smith, 1985). Calcium is an important component of plant cell walls and membranes. Increasing leaf Ca concentrations with time may have been a consequence of Ca accumulation in the leaves, and the low mobility of Ca in plant tissues. For the mango cultivars used in this study, the fruiting period in south Florida occurs between March and July. Daytime temperatures averages over the three years of the study in March, April, May, June, and July were 20.92°C, 23.33°C, 25.27°C, 26.73°C, and 27.21°C, respectively. Thus, high transpiration rates, which probably occurred during the fruiting period because of high temperatures, could have resulted in the high Ca concentrations observed in the leaves. The high Ca concentrations observed in the leaves indicate that the mango trees were probably adequately supplied with Ca during the study.

In the fruit, Ca concentrations decreased with age. The lowest Ca concentrations were observed in ripe fruit. Fruit Ca was constantly maintained below 1%, which is lower than the 2.05% and 2.85% for 'Tommy' and 'Irwin', respectively, reported by Laborem (1979). 'Van Dyke' and 'Tommy Atkins' had lower fruit Ca concentrations than 'Irwin', but foliar Ca was lower in 'Irwin' than in the other two cultivars. In the ripe fruit, the ratios of leaf to fruit Ca concentrations were 23.50, 30.03, and 37.20 for 'Irwin', 'Tommy Atkins', and 'Van Dyke', respectively. When the fruit were first sampled, the ratios were 7.0, 8.68, and 22.03 for the respective cultivars. These observations indicate that nutrient absorption may be cultivar related. Tyler and Lorenz (1963) conducted a study of nutrient absorption in 'Persian', 'Honeydew',

'Creenshaw', and 'Cantaloupe PMR450' muskmelons. The fruit Ca concentration decreased in 'Cantaloupe PMR450' and 'Crenshaw' melons throughout fruit ontogeny, whereas fruit Ca levels either increased or remained stable in 'Persian' or 'Honeydew' melons, respectively. In a study of the Ca concentration during melon (*Cucumis melo* L., var. Maestro) fruit development, Bernadac et al. (1996) also recorded significant declines in fruit Ca concentrations throughout fruit ontogeny. The flesh Ca concentration of the control fruit (with no calcium applied) decreased from 0.26 g 100 g^{-1} DM in 20-day-old fruit to 0.07 g 100 g^{-1} DM in 37-day-old fruit (Bernadac et al., 1996). Calcium transport within tissues occurs in the xylem with the transpiration stream. Consequently, Ca is mainly found in actively transpiring organs such as leaves and young fruits. Moreover, the xylem is more developed in leaves than in fruit (Esau, 1977). In addition, Ca does not translocate readily from old organs to younger ones. Thus, leaves are a stronger sink for Ca than fruit, possibly explaining the greater accumulation of Ca in the leaves than in the fruit of mango.

Magnesium concentrations in the leaves were higher than those observed in the fruit in this study. In general, Mg concentrations in the leaves and fruit significantly decreased as the fruit developed. Also, Mg concentrations in the leaves and fruit were lowest when the fruit were ripe. Magnesium is a component of the chlorophyll molecule (Bidwell, 1979). Since leaves generally have a higher chlorophyll concentration than fruit, Mg concentrations would be expected to be higher in leaves than in fruit.

As shown in Table 4-2, the leaves contained considerably higher concentrations of micronutrients than the fruit. On the first sampling date, the order of magnitude of micronutrient concentrations in the fruit was Fe > Zn > Cu > B > Mn, and Zn > Mn > Fe > Cu > B in the leaves. However, when the fruit were ripe, the order of magnitude of these nutrients was Fe > Cu > Zn > B > Mn in the fruit, and Fe > Cu > Zn > Mn > B in the leaves. The displacement observed between Cu and Zn

Table 4-2. Concentrations of micronutrients in mango leaves and fruit at the beginning of the fruiting period and at when fruit were ripe.

Organ sampled	Stage of fruit devp.	mg Kg^{-1} DW[z]				
		Zn	Cu	Mn	Fe	B
Fruit	5-week-old	37.90	26.02	12.75	43.57	15.76
	Ripe	11.88	21.20	5.00	25.51	9.43
Leaves	5-week-old	202.12	126.24	157.32	145.83	35.53
	Ripe	240.00	261.41	181.38	298.92	39.30

[z]: DW= Dry weight

may not necessarily be due to actual metabolic activity in the plant but may be related to spraying practices to protect fruit from fungal diseases, especially anthracnose, as fruit ripening approached. In Florida, fruit maturity and/or ripening of 'Irwin', 'Tommy Atkins', and 'Van Dyke' mangos generally occurs around June. Daytime temperature and rainfall from 1994 to 1996 averaged 26.73°C and 382.75 mm, respectively. Such conditions are ideal for fungal proliferation. Kocide, a Cu-based product, is among the most widely used fungicides in mango orchards to control anthracnose. Thus, Cu levels may have increased in the leaves and fruit near fruit ripening as a result of fungicide application.

Among all the microelements, mango requires B in the lowest concentrations. Micronutrient concentrations, except for B (Table 4-2), exceeded de Laroussilhe's (1980) recommendations regarding the ideal concentrations of micronutrients in mango leaves. Mengel and Kirkby (1982) pointed out that B availability is reduced with increased soil pH. This may explain the low B concentration observed in the fruit. Additionally, B is transported in the xylem, which is generally rudimentary in fruit (Esau, 1977). Thus, the leaves are presumably a stronger sink for B than the fruit.

Conclusions

Leaf N, P, and K concentrations fluctuated little over time as illustrated in Figures 4-1 to 4-3. This suggests that leaf samples may be collected at any time during fruit ontogeny for determination of N, P, and K concentrations in mango leaves. Calcium accumulated in mango leaves and decreased in fruit throughout fruit ontogeny. Unlike Ca, Mg decreased both in the leaves and fruit during fruit development. Ripe 'Irwin', 'Tommy Atkins', and 'Van Dyke' mango fruit had similar concentrations of mineral elements. In general, younger fruit had higher concentrations of mineral elements than ripe fruit. Fruit nutrient concentrations generally decreased with time and, consequently, with fruit size, probably as a result of dilution due to fruit enlargement. Nutrient deficiencies or imbalances, when present, probably occur early during fruit ontogeny, apparently during the cell enlargement phase of the fruit, which occurs from the fourth WAFS until fruit maturity. Mango leaves have a higher number of functional stomata and a more efficient xylem than mango fruit. Mango fruit are not as strong of a sink as the leaves for mineral elements such as Ca and B transported in the xylem via the transpiration stream. To prevent mineral deficiencies in fruit, mineral elements should be supplied in sufficient amounts before fruit enter the phase of accelerated growth, preferably before fruit set. It may be possible to alter the relative sink strength of the fruit by reducing the transpiration rate of the leaves. A possible method of accomplishing this would be with the use of antitranspirant sprays. Increasing the sink strength of the fruit relative to that of the leaves may help to eliminate fruit disorders in mango caused by nutrient deficiencies.

CHAPTER V

EFFECTS OF FOLIARLY APPLIED CALCIUM ON MINERAL NUTRIENT CONCENTRATIONS AND INTERNAL BREAKDOWN OF 'IRWIN', 'TOMMY ATKINS', AND 'VAN DYKE' MANGO FRUIT

Introduction

Internal breakdown is a physiological disorder of mango fruit that is characterized by a disequilibrium in the ripening of the fruit, i.e. part of the pulp ripens at a faster rate than the remaining mesocarp. The disorder is undetectable unless the fruit is cut open. Internal breakdown is alternately referred to as 'jelly seed', 'soft nose', 'spongy tissue', or 'stem end cavity' (SEC), depending on the location of the tissue breakdown within the fruit. The disorder has been reported to cause severe yield losses in several mango producing regions such as India, Florida, the Canary Islands, Australia, Malaysia, Brazil, and Venezuela.

The cause of internal breakdown of mango has been attributed to a variety of factors including environmental variables (Gunjate et al., 1982; Lad et al., 1992) and orchard management practices (Malo and Campbell, 1978). However, the factor most often associated with internal breakdown in mango has been Ca deficiency (Shear, 1975; Young, 1957). Calcium deficiency has been related to a number of physiological disorders in other crop species, including bitter pit of apples (Bangerth, 1979; Shear, 1975), blossom end rot of tomatoes, and blackheart of celery (Maynard , 1979; Shear, 1975).

The occurrence of internal breakdown in mango fruit has previously been related to low plant Ca concentrations (Young et al., 1965; Burdon et al. 1991).

However, in previous studies relating internal breakdown to plant Ca concentrations, Ca concentrations were determined only in mature fruit or leaves. There are no reports relating fruit mineral concentrations throughout fruit ontogeny to the incidence of internal breakdown. Young et al., (1965) observed that the intensity of 'soft nose' in 'Kent' mango fruit declined with increased Ca concentrations, whereas high concentrations of leaf N were associated with an increased incidence of the disorder in ripe fruit. Burdon et al. (1991) compared the mineral composition of immature and mature green fruit of 'Kent', a cultivar susceptible to soft nose, to that of 'Beverly', a cultivar tolerant to soft nose. In that study, the Ca and Mg concentrations in disordered 'Kent' fruit were lower than that of the healthy 'Kent' fruit. Also, Ca and Mg concentrations in disordered 'Kent' mangoes were similar to concentrations in healthy 'Beverly' fruit. Finally, the Ca concentration in the soft nose-affected tissue was lower than that in healthy tissue in the same fruit. In a study of 'Alphonso' mangoes, dipping fruit in 5,000 mg/L and 20,000 mg/L $CaCl_2$ or $Ca(NO_3)_2$ increased fruit Ca concentration and decreased the incidence of spongy tissue (Gunjate et al., 1979). However, in another study there were no differences in Ca, Na, or Mg between healthy and disordered fruit of 'Alphonso' (Krishnamurthy 1981). In that study, healthy fruit had lower P and K concentrations than disordered fruit, and the concentrations of acetaldehyde, pyruvic acid, and α-ketoglutaric acid were higher in affected fruit than in the healthy fruit.

If Ca deficiency is responsible for internal breakdown of mango, the deficiency is probably established at a specific moment of fruit development. Calcium is an important stabilizing component of cell membranes (Hepler and Wayne, 1985). Therefore, it is most likely that Ca deficiency at an early stage of fruit ontogeny, when cells are rapidly developing, would result in internal breakdown. The application of Ca to the fruit may increase fruit Ca concentrations and reduce or prevent the occurrence of the disorder. The purpose of this study was to test the effects of foliar applications of

Ca on the concentrations of Ca and other mineral elements in the fruit, and on the incidence of internal breakdown in 'Irwin', 'Tommy Atkins', and 'Van Dyke' mangoes.

Materials and Methods

Location. Experiments were conducted in commercial orchards in south Dade County (25.36°N and 80.21°W) during the 1994-1995 and 1995-1996 fruiting seasons. The orchards were established on Krome very gravelly loam soil (loamy, skeletal, carbonatic, hyperthermic Lithic Udorthents). These soils are 0-27.50 cm deep and made of very gravelly loam or weathered bedrock. They contain 15-20% clay and have a pH of 7.4-8.4 (USDA, 1996). Daytime temperatures during the experimental period averaged 24.3°C in 1994-1995 and 23.6°C in 1995-1996. Rainfall totals were 911.0 mm in 1994-1995 and 894.5 mm in 1995-1996, with a peak in June during both experimental periods.

Plant materials. Trees of 'Irwin', 'Tommy Atkins', and 'Van Dyke' mango grafted on 'Turpentine' rootstock from two orchards were used in the 1994-1995 experiment. Orchard I included 15-year-old 'Van Dyke' trees, whereas 'Irwin' and 'Tommy Atkins' trees of orchard II were 40 years old. Orchard III, with only 30-year-old 'Tommy Atkins' trees, was used in 1995-1996.

In 1994-1995, 'Van Dyke' trees in orchard I received 630 kg/ha of 3-8-12 (N-P-K) in September 1994, and three applications of 5.23 kg/ha, 7.61 kg/ha, and 9.55 kg/ha of Sequestrene-Fe (Geigy 138, 5% Fe) in September 1994, January 1995, and May 1995, respectively. Trees were hedged and topped in September. Another fertilizer application consisting of 646.59 kg/ha of 6-0-19 (N-P-K) was made in April 1995. During the same season, 'Tommy Atkins' and' Irwin' trees from orchard II were given only 568 kg/ha of 6-0-19 (N-P-K), as the orchard was scheduled to be sold.

Orchard III received no fertilizer in 1996. However, 'Tommy Atkins' trees in that orchard had received 568 kg/ha of 6-0-19 (N-P-K) in March 1995, 9.94 kg/ha of Sequestrene-Fe (Geigy 138, 5% Fe) in August 1995, 852.27 kg/ha of 3-8-12 (N-P-K) and 19.32 kg/ha of Sequestrene-Fe (Geigy 138, 5% Fe) in September 1995, and 19.32 kg/ha of Sequestrene-Zn (Geigy 138, 14% Zn), and 19.32 kg/ha of Sequestrene-Mn (Geigy 138, 12% Mn) in December 1995.

A split-plot design was used in the 1994-1995 trial in which cultivars were the main plots and $CaCl_2$ concentrations were the subplots. Calcium chloride was applied at 0, 4.6, 9.2, or 18.4 g/L to 'Irwin', 'Tommy Atkins', and 'Van Dyke' trees. Each treatment was applied every 2 weeks to three single-tree replications beginning 4 weeks after fruit set (WAFS) until the fruit were ripe. Each tree received approximately 10 L of solution. Freeway (Loveland Industries Inc., CO USA), an organo-silicone wetter/spreader/penetrant was used as a surfactant at 0.5 ml/L. A randomized complete block design was used in 1995-1996 and treatments consisted of four concentrations of Cab'Y (Stoller Chemical Company of Florida, Inc., Eustis, FL, USA) and Packhard (Micro Flo Company, Lakeland, FL, USA). Cab'Y contains 100 g/L Ca whereas Packhard has 80 g/L Ca. Both products contain 5 g/L of B. In the 1995-1996 trial, Cab'Y and Packhard were used at 0, 36, 54, or 72 g/L. Each treatment was applied every week to four single-tree replications beginning 4 WAFS until the fruit were ripe. Each tree received approximately 10 L of solution. LI-700 (Loveland Industries Inc., Greenlay, CO, USA) was used as penetrant at 0.5 ml/L. During both years, foliar sprays were applied with a hand-gun sprayer.

Sampling. In the 1994-1995 trial, leaf and fruit samples were collected every 2 weeks from each tree, starting 4 WAFS. Samples were collected in the early morning of the day before $CaCl_2$ solutions were sprayed. Five mature, healthy leaves and 3 fruit were collected from each tree at each sampling date from the middle portion of a fruit-bearing branch. Sampling continued until the fruit were ripe. Fruit were considered

ripe when the mesocarp was soft enough to allow consumption as a fresh fruit. In the 1995-1996 trial, leaf and fruit samples were collected on a weekly basis in the morning, and treatments were sprayed after samples were collected. Samples were placed in paper bags and shaded to prevent water loss that could result from prolonged exposure to the sun during sampling and transportation from the field to the laboratory.

Processing of samples. Fruit and leaf samples were washed in a 10 ml/L detergent solution, rinsed in tap water, washed in 0.6 M HCl, and rinsed twice in distilled water as described by Schaffer et al. (1988). After washing, the fruit were weighed. Leaf samples were oven dried at 70°C for 48 hours and ground in a cyclone mill (UDY Corp., Fort Collins, CO). Fruit samples were oven dried for 48-120 hours, depending upon fruit size.

For K, P, Ca, Mg, Zn, Cu, Mn, Fe, and B determination, 1 g of ground tissue was weighed in a 40 mL high-form porcelain crucible (Fischer Scientific, Pittsburgh, PA) and ashed at 500°C in a muffle furnace (Furnatrol FA 1730, Barnstead/Thermolyne, Dubuque, IA). The ashed sample was digested with 5 ml of 6 M HCl and brought to 50 mL with distilled water in a polyethylene volumetric flask. The preparation was shaken and filtered through Whatman #1 filter paper into a 20-ml scintillation vial. Polyethylene vials were used instead of glass so that there was no borosilicate to interfere with B determinations.

For N determinations, 0.2 g of ground tissue was weighed into a 100-ml digestion tube to which 2 g of Kjeldahl mixture and 5 ml of concentrated H_2SO_4 were added. Glass funnels were placed on the tubes and tubes and funnels were placed on a preheated aluminum digestion block set at 250°C for 1 hour. The temperature of the digestion block was raised to 380°C for an additional 3-hour period. After cooling, 5 ml of distilled water were added to each tube and the preparation was agitated with a vortex mixer. The digested material was transferred to a 100 ml volumetric flask, and the content was brought to 100 ml with distilled water and allowed to cool. The

preparation was vigorously mixed and filtered through Whatman #1 filter paper into a 20-ml polyethyelene scintillation vial (Hanlon et al., 1994). Nitrogen concentrations in fruit and leaves were determined by the Total Kjeldahl Nitrogen (TKN) method, and K, P, Ca, Mg, Zn, Cu, Mn, Fe, and B were determined by inductively coupled argon plasma spectroscopy (ICAP) (Hanlon et al., 1994).

Determination of fruit fresh weight and internal breakdown. Fruit fresh weight was determined by individually weighing fruit immediately after collection from the orchards. The presence of soft nose, jelly seed, and SEC was determined after cutting open the fruit samples. A transverse cut was made first at the proximal end of each fruit, immediately under the skin to expose the tissues in the peduncular extension and to assess the presence of SEC. Two additional lateral cuts were then made on each of the wider, flat sides of the stone to expose the interior of the fruit and detect the presence of either jelly seed or soft nose.

Statistical analysis was performed with SAS 6.02 (SAS Inst., Cary, NC), and cultivar means for N, P, K, Ca, Mg, Zn, Cu, Mn, Fe, and B concentrations as well as for the percentage of disordered fruit were compared by a Duncan's multiple range test at $P<0.05$.

Results

Effects of Ca treatments on mineral element concentrations

1994-1995 trial. During the 1994-1995 season, there were no significant effects of the application rates of $CaCl_2$ on N, P, K, Ca, Mg, Zn, Cu, Mn, Fe, or B concentrations in 'Irwin', 'Tommy Atkins', or 'Van Dyke' fruit. Table 5-1 shows the concentrations of these mineral elements in treated and untreated fruit of the three cultivars. In the table, control refers to the untreated trees, whereas $CaCl_2$ represents the combination of the 4.6, 9.2, and 18.4 g/L $CaCl_2$ treatments applied to the fruit

every two weeks. For each cultivar, the concentrations of the individual mineral elements in the control fruit were similar to the concentrations observed in the fruit that were sprayed with $CaCl_2$, regardless of the concentration of the solution (Table 5-1).

Table 5-1. Mineral element concentrations in 'Irwin', 'Tommy Atkins', and 'Van Dyke' mango fruit sprayed with $CaCl_2$ at two-week intervals Each value is the mean of 12 (untreated) or 36 (treated) fruit. Treated = average of the 4.6, 9.2, and 18.4 g/L $CaCl_2$ treatments. There were no significant differences between treated and untreated fruit at $P<0.05$.

Mineral element	Cultivars (1995)					
	'Irwin'		'Tommy Atkins'		'Van Dyke'	
	Control	$CaCl_2$	Control	$CaCl_2$	Control	$CaCl_2$
	g 100 g^{-1} DW[z]					
N	0.94	1.04	0.94	1.05	0.96	0.97
P	0.16	0.17	0.19	0.20	0.16	0.16
K	1.46	1.5	1.39	1.52	1.29	1.37
Ca	0.36	0.37	0.33	0.35	0.28	0.29
Mg	0.12	0.15	0.11	0.11	0.10	0.11
	mg kg^{-1} DW					
Zn	17.24	18.37	15.67	19.35	15.91	16.19
Cu	20.03	21.16	19.47	22.62	20.91	23.33
Mn	7.64	8.03	5.94	5.71	4.77	5.28
Fe	19.52	18.95	17.92	19.64	20.50	21.97
B	9.95	10.48	11.68	12.40	11.93	11.97

[z]: DW= Dry weight

The mineral element concentrations in the fruit varied among cultivars and with the application time. In general, fruit N concentrations declined over time and reached

their lowest point when the fruit were ripe (Table 5-2). When the fruit were ripe, the N concentrations were 52.35%, 67.54%, and 58.18% of the concentrations observed 8 WAFS in 'Irwin', 'Tommy Atkins', and 'Van Dyke', respectively. The N concentration at 8 WAFS was higher in 'Tommy Atkins' than in 'Irwin' or 'Van Dyke'.

The N concentration was higher in 'Tommy Atkins' than in 'Irwin' or 'Van Dyke' fruit at 8 WAFS, but declined rapidly between 8 and 10 WAFS. At 12 and 16 WAFS, the fruit N concentrations were similar among the three cultivars, but N levels declined between 12 and 14 WAFS in 'Irwin' and 'Van Dyke'. At 14 WAFS, the fruit N concentrations was again significantly higher in 'Tommy Atkins' than in 'Irwin' or 'Van Dyke', and the fruit N concentrations in 'Irwin' were higher than in 'Van Dyke'. When the fruit were ripe, the N concentration in 'Irwin' fruit was higher than in 'Tommy Atkins' fruit, but there were no differences in fruit N concentrations between 'Irwin' and 'Van Dyke', and between 'Tommy Atkins' and 'Van Dyke' fruit.

Phosphorus concentrations were higher in 'Tommy Atkins' than in 'Irwin' or 'Van Dyke' fruit throughout most of the fruit development period (Table 5-3). The P concentrations remained relatively high through the first three sampling dates, then declined sharply between 8 and 10 WAFS. In 'Irwin', the fruit P concentration was lowest (0.09 g 100 g^{-1}) at 16 WAFS, i.e. 2 weeks before the fruit were ripe, whereas in 'Tommy Atkins' and 'Van Dyke' the minimum level of fruit P was reached at 12 WAFS. On the first sampling date, the P concentration was lower in 'Van Dyke' and 'Irwin' fruit than in 'Tommy Atkins' fruit. However, there were no significant differences in fruit P concentrations between ripe 'Tommy Atkins' and ripe 'Van Dyke' fruit. Ripe 'Irwin' fruit had a lower P concentration than ripe 'Tommy Atkins' or 'Van Dyke' fruit.

The K concentrations in the fruit fluctuated until the fruit were ripe. On the first sampling date, the K concentration was lowest in 'Van Dyke', intermediate in 'Irwin',

Table 5-2. Nitrogen concentrations in 'Irwin', 'Tommy Atkins', and 'Van Dyke' fruit sprayed with $CaCl_2$ at 2-week intervals. Each value is the mean of 36 fruit. Means with the same letter are not significantly different at $P<0.05$. (T.A.='Tommy Atkins'; V.D.= 'Van Dyke').

Cultivar	Weeks after fruit set (1995)					
	8	10	12	14	16	18
	N (g 100 g^{-1} DW)					
'Irwin'	1.49 b	1.15 a	0.91 a	0.78 b	0.75 a	0.71 a
'T. A'	1.91 a	0.96 b	0.93 a	0.90 a	0.81 a	0.62 b
'V. D'	1.65 b	1.14 a	0.89 a	0.69 c	0.76 a	0.69 ab

Table 5-3. Phosphorus concentrations in 'Irwin', 'Tommy Atkins', and 'Van Dyke' mango fruit sprayed with $CaCl_2$ at 2-week intervals. Each value is the mean of 36 fruit. Means with the same letter are not significantly different at $P<0.05$. (T.A.= 'Tommy Atkins'; V.D.= 'Van Dyke').

Cultivar.	Weeks after fruit set (1995)							
	4	6	8	10	12	14	16	18
	P (g 100 g^{-1} DW)							
'Irwin'	0.30 b	0.20 c	0.27 a	0.12 b	0.11 b	0.11 b	0.09 c	0.12 b
'T. A'	0.37 a	0.27 a	0.25 a	0.14 a	0.13 a	0.14 a	0.13 a	0.14 a
'V. D'	0.29 c	0.24 b	0.20 b	0.13ab	0.11 b	0.11 b	0.11 b	0.13 a

and highest in 'Tommy Atkins' fruit (Table 5-4). In general, 'Van Dyke' fruit contained lower K concentrations than 'Irwin' or 'Tommy Atkins' fruit. Despite the significant differences in fruit K concentrations among the cultivars observed on the first sampling date, ripe fruit of the three cultivars did not differ significantly in K

concentrations. The lowest fruit K concentrations were observed 16 WAFS in 'Irwin', and 10 and 12 WAFS in 'Tommy Atkins' and 'Van Dyke', respectively. When the fruit were ripe, fruit K concentrations had declined by 12.78% compared to the concentrations observed at 4 WAFS in 'Irwin', and by 24.38% at 4 WAFS in 'Tommy Atkins'. The K concentration in ripe 'Van Dyke' fruit was identical to the K concentration observed on the first sampling date. When the fruit had ripened, there were no differences in fruit K concentrations among the three cultivars.

Fruit Ca concentrations fluctuated with time throughout fruit ontogeny. In 'Irwin', the fruit Ca continuously declined from 4 WAFS until the fruit were ripe. In 'Tommy Atkins' fruit, there was a rapid decrease in the fruit Ca concentrations at 10 WAFS. The fruit Ca levels then remained fairly constant until 16 WAFS (Table 5-5). 'Van Dyke' fruit also exhibited a rapid decrease in Ca concentration at 10 WAFS, which was followed by a continuous decline until 16 WAFS, thereafter increasing more than 50% in ripe fruit. The fruit Ca concentration in 'Irwin' (0.68 g 100 g^{-1}) was similar to that of 'Tommy Atkins' (0.57 g 100 g^{-1}) at 4 WAFS, but young 'Irwin' fruit contained higher Ca concentrations than 'Van Dyke' fruit (0.53 g 100 g^{-1}) at that sampling date. The fruit Ca concentrations were generally similar for 'Irwin' and 'Tommy Atkins' throughout most of the fruit development period. However, significant differences in fruit Ca concentrations were recorded at 6 and 12 WAFS (Table 5-5). Fruit Ca concentrations were not significantly different among the cultivars at 8 WAFS and when the fruit were ripe.

Magnesium concentrations in 'Irwin' fruit were generally higher than in 'Tommy Atkins' or 'Van Dyke' fruit, whereas no differences in fruit Mg existed between 'Tommy Atkins' and 'Van Dyke' throughout most of the fruit development period (Table 5-6). Magnesium concentrations declined about 35% through the first 10 WAFS and were stable thereafter. There were no differences in fruit Mg concentrations among the three cultivars at 6 and 8 WAFS. At 10 and 14 WAFS, the concentrations of

Table 5-4. Potassium concentrations in 'Irwin', 'Tommy Atkins', and 'Van Dyke' mango fruit sprayed with $CaCl_2$ at two-week intervals. Each value is the mean of 36 fruit. Means with the same letter are not significantly different at $P<0.05$. (T.A.= 'Tommy Atkins'; V.D.= 'Van Dyke').

Cultivar	Weeks after fruit set (1995)							
	4	6	8	10	12	14	16	18
	K (g 100 g^{-1} DW)							
'Irwin'	1.80 b	1.46 a	1.48 a	1.56 a	1.38 a	1.48 a	1.22 b	1.57 a
'T. A'	2.01 a	1.49 a	1.36 a	1.33 b	1.34 a	1.42 a	1.40 a	1.52 a
'V. D'	1.50 c	1.25 b	1.55 a	1.26 b	1.19 b	1.23 b	1.30 ab	1.50 a

Table 5-5. Calcium concentrations in 'Irwin', 'Tommy Atkins', and 'Van Dyke' mango fruit sprayed with $CaCl_2$ at two-week intervals. Each value is the mean of 36 fruit. Means with the same letter are not significantly different at $P<0.05$. (T.A.= 'Tommy Atkins'; V.D.= 'Van Dyke').

Cultivar	Weeks after fruit set (1995)							
	4	6	8	10	12	14	16	18
	Ca (g 100 g^{-1} DW)							
'Irwin'	0.68 a	0.50 a	0.40 a	0.35 a	0.31 a	0.26 a	0.24 a	0.22 a
'T. A'	0.57 ab	0.42 b	0.38 a	0.28 b	0.28 a	0.27 a	0.20 a	0.21 a
'V. D'	0.53 b	0.37 b	0.40 a	0.24 c	0.20 b	0.18 b	0.14 b	0.22 a

Mg were significantly higher in 'Irwin' fruit than in 'Tommy Atkins' or 'Van Dyke' fruit, but no differences in Mg concentrations existed between 'Tommy Atkins' and 'Van Dyke' fruit at those sampling dates.

Fruit Mg concentrations slightly increased before the fruit were ripe in all three cultivars. At the end of the sampling period, the Mg concentration in ripe 'Irwin' fruit was significantly higher than in 'Tommy Atkins' or 'Van Dyke' fruit, but no differences in Mg concentrations existed between 'Tommy Atkins' and 'Van Dyke' fruit at that sampling date. When the fruit were ripe, the Mg concentrations had declined by 29.41% from the concentration recorded on the first sampling date for 'Irwin' fruit, and by 28.57% and 35.71% from the concentrations first observed in 'Tommy Atkins' and 'Van Dyke' fruit, respectively.

There were no significant differences in Zn, Cu, Fe, and B concentrations among the three cultivars (data not shown). The concentrations of Zn, Cu, Mn, Fe, and B declined with time in the fruit of all three cultivars, and the lowest levels were observed 2 weeks before the fruit were ripe.

Significant differences in fruit Mn concentrations were observed among the cultivars. Except for 8 WAFS and when the fruit were ripe, the fruit Mn concentration was highest in 'Irwin', lowest in 'Van Dyke', and intermediate in 'Tommy Atkins' fruit throughout the fruit development period (Table 5-7). There was no significant difference in fruit Mn concentrations between 'Tommy Atkins' and 'Van Dyke' at 8 WAFS. In all three cultivars, the fruit Mn concentration was lowest at 16 WAFS, i.e. 2 weeks before the fruit were ripe. Between 16 and 18 WAFS, Mn concentrations increased significantly in all three cultivars and the final Mn concentrations were not significantly different among the cultivars.

1995-1996 trial. The effects of Cab'Y and Packhard applications on mineral element concentrations in 'Tommy Atkins' fruit varied with the time of application and the product. For example, at 4 WAFS, Cab'Y and Packhard significantly affected the fruit N concentration. At that time, N concentrations in the fruit were 1.88 g 100 g^{-1} in the control (0 g/L Ca) treatment, and 1.90, 1.95, and 1.75 g 100 g^{-1} in the 3.6, 5.4, and 7.2 g/L Ca Cab'Y treatments, respectively.

Table 5-6. Magnesium concentrations in 'Irwin', 'Tommy Atkins', and 'Van Dyke' mango fruit sprayed with $CaCl_2$ at 2-week intervals. Each value is the mean of 36 fruit. Means with the same letter are not significantly different at $P<0.05$. (T.A.= 'Tommy Atkins'; V.D.= 'Van Dyke').

Cultivars	Weeks after fruit set (1995)							
	4	6	8	10	12	14	16	18
	Mg (g 100 g^{-1} DW)							
'Irwin'	0.17 a	0.13 a	0.14 a	0.11 a	0.10 a	0.11 a	0.10 a	0.12 a
'T. A'	0.14 b	0.13 a	0.13 a	0.09 b	0.09 ab	0.09 b	0.09 ab	0.10 b
'V. D'	0.14 b	0.12 a	0.13 a	0.09 b	0.08b	0.08 b	0.08 b	0.09 b

Table 5-7. Concentrations of Mn in 'Irwin', 'Tommy Atkins', and 'Van Dyke' mango fruit sprayed with $CaCl_2$ at 2-week intervals. Each value is the mean of 36 fruit. Means with the same letter are not different at $P<0.05$. (T.A.='Tommy Atkins'; V.D.= 'Van Dyke').

Cultivars	Weeks after fruit set (1995)					
	8	10	12	14	16	18
	Mn (mg kg^{-1} DW)					
'Irwin'	12.72 a	9.42 a	7.42 a	6.96 a	5.13 a	5.96 a
'T.A.'	7.39 b	7.00 b	5.96 b	5.42 b	4.08 b	4.75 a
'V.D.'	8.89 b	5.83 c	4.17 c	3.92 c	3.17 c	4.96 a

Nitrogen concentrations in fruit sprayed with Packhard were 2.05, 1.73, and 1.59 g 100 g^{-1} with the 3.6, 5.4, and 7.2 g/L Ca treatments, respectively. There was a significant quadratic relationship between Cab'Y and Packhard and N concentration in the fruit. The quadratic regression equation for Cab'Y was $Y=1.68 + 0.24x - 0.06x^2$ ($r^2=0.74$) and for Packhard $Y= 1.71 + 0.28x - 0.08x^2$ ($r^2=0.74$). For Cab'Y, there

was a significant, but weaker, relationship between the applied concentrations and the fruit N concentration 9 WAFS (r^2=0.41). Packhard concentration was significantly related to fruit N concentration later during fruit ontogeny, i.e. 11 WAFS (r^2=0.41) and 13 WAFS (r^2=0.34).

Cab'Y had no effect on the fruit P concentrations at any sampling date. A significant effect of Packhard on fruit P concentrations was observed only 4 WAFS. At that sampling date, the P concentrations in the fruit were 0.23, 0.26, 0.23, and 0.20 g 100 g^{-1} for 0, 3.6, 5.4, and 7.2 g/L Ca treatments, respectively, (r^2=0.38 for the linear relationship and r^2=0.69 for the quadratic relationship).

There was a significant relationship between Cab'Y concentrations and fruit K concentrations at 4 WAFS ($Y = 1.46 + 0.28x - 0.05x^2$; r^2=0.40). The fruit K concentrations were 1.69 g 100 g^{-1} for the 0 g/L Ca treatment, and 1.83, 1.82, and 1.76 g 100 g^{-1} for the 3.6, 5.4, and 7.2 g/L Ca treatments, respectively. Packhard concentrations were also significantly related to the fruit K concentration on the first sampling date, i.e. 4 WAFS ($Y = 1.56 + 0.19x-0.05x^2$; r^2=0.37), and also when the fruit were ripe ($Y = 1.40 - 0.23x+0.07x^2$; r^2=0.41). At 4 WAFS, the highest fruit K concentration (1.81 g 100 g^{-1}) was observed for the 3.6 g/L Ca treatment compared to 1.60 g 100 g^{-1} observed with the 7.2 g/L Ca treatment. However, in the ripe fruit, the highest K concentration (1.66 g 100 g^{-1}) was recorded for the 7.2 g/L Ca treatment, whereas the lowest K level (1.23 g 100 g^{-1}) was observed in the control treatment.

A significant effect of Cab'Y concentration on fruit Ca concentration was observed only on the last sampling date, whereas Packhard concentration was significantly related to fruit Ca concentrations at 4 WAFS and when the fruit were ripe. On the first sampling date, i.e. 4 WAFS, only Packhard concentration was significantly related to fruit Ca concentration ($Y = 0.47 - 0.10x + 0.02x^2$; r^2=0.40). On that sampling date, the fruit Ca concentration was 0.39 g 100 g^{-1} for the control treatment, whereas the fruit Ca concentrations for the 3.6, 5.4, and 7.2 g/L Ca treatments were

0.37, 0.42, and 0.42 g 100 g^{-1}, respectively. As the fruit developed, the strength of the relationship between Packhard concentration and fruit Ca concentration weakened (r^2=0.40 at 4 WAFS compared to r^2=0.38 when the fruit were ripe). The strength of the relationship between Cab'Y concentration and fruit Ca concentration was weaker (r^2=0.28) than that of Packhard when the fruit were ripe. In the ripe fruit, the lowest fruit Ca concentration (0.11 g 100 g^{-1}) was observed with the 0 g/L Ca treatment, whereas the highest fruit Ca concentrations (0.15 g 100 g^{-1} for Cab'Y) and (0.17 g 100 g^{-1} for Packhard) were observed with the 7.2 g/L Ca treatment.

Fruit Mg concentrations were significantly related to the concentrations of Cab'Y and Packhard only during the early stages of fruit ontogeny. Cab'Y concentrations were related to fruit Mg concentrations at 6 WAFS (linear regression equation: $Y= 0.14 - 0.008x$; r^2=0.36, quadratic regression equation: $Y= 0.14 - 0.008x - 0.0002x^2$; r^2=0.36). At that sampling date, the Mg concentrations in the fruit were 0.14 g 100 g^{-1} for the 0 g/L Ca, and 0.12, 0.11, and 0.11 g 100 g^{-1} for the 3.6, 5.4, and 7.2 g/L Ca treatments applied as Cab'Y. There was a significant relationship between Packhard application rates and fruit Mg concentrations only at 4 WAFS ($Y= 0.18 - 0.02x + 0.004x^2$; r^2=0.49). The highest Mg concentration (0.14 g 100 g^{-1}) was observed in the control treatment, and the fruit Mg concentration declined with increasing Ca concentrations, e.g. 0.13, 0.13, and 0.12 g 100 g^{-1} for the 3.6, 5.4, and 7.2 g/L Ca treatments, respectively, applied as Packhard.

Cab'Y and Packhard concentrations were significantly related to fruit Zn concentrations at 4 and 13 WAFS. The relationship between Cab'Y and fruit Zn concentration was stronger in the mature fruit (r^2=0.47) than in the younger fruit (r^2=0.37). The quadratic relationship between Packhard concentrations and the fruit Zn concentrations was stronger at 4 WAFS ($Y= 45.38 - 10.08x + 1.63x^2$; r^2=0.60) than at 13 WAFS ($Y= 6.93 + 2.68x - 0.41x^2$; r^2=0.52).

Cab'Y concentrations were significantly related to fruit Cu concentrations only at 4 WAFS and when the fruit were ripe. The relationship between Cab'Y concentrations and fruit Cu concentrations was stronger at 4 WAFS ($Y = 38.34 - 9.96x + 1.59x^2$; $r^2=0.50$) compared to when the fruit were ripe ($Y = 26.11 - 3.72x + 1.22x^2$; $r^2=0.40$). At 4 WAFS, the highest fruit Cu concentration (30.75 g $100g^{-1}$) was observed with the 0 g/L Ca treatment, and the Cu levels in the fruit declined with increasing Ca rates. Packhard concentrations were related to the fruit Cu concentrations for longer period time than Cab'Y.

A relationship between Cab'Y concentrations and fruit Mn concentration was observed only at 10 WAFS and when the fruit were ripe. On both occasions, the coefficient of determination of Cab'Y concentration versus fruit Mn concentration was similar ($r^2= 0.36$). Packhard concentrations were significantly related to fruit Mn concentrations at 4 WAFS ($r^2=0.44$) and 13 WAFS ($r^2=0.46$).

The Fe concentrations in the fruit were affected by Cab'Y application rates at 4 and 5 WAFS, whereas Packhard concentration was significantly related to fruit Fe concentration from 4 to 7 WAFS. For Cab'Y, the strength of the relationship declined at 5 WAFS to $r^2=0.48$ from $r^2=0.51$ recorded at 4 WAFS. A similar decline over time was observed for Packhard at 4 WAFS ($r^2=0.69$) and 7 WAFS ($r^2=0.43$).

The fruit B concentrations were significantly related to Cab'Y (Table 5-8) and Packhard concentrations (Table 5-9) throughout most of the fruit development period. In general, the coefficients of determination were higher for Packhard than for Cab'Y on every sampling date, except when the fruit were ripe (14 WAFS).

Effects of Ca treatments on incidence of internal breakdown

1994-1995 trial. For non-control trees, there were no significant effects of $CaCl_2$ on the incidence of internal breakdown applied at any rate at any time during fruit ontogeny in 1995. Therefore, for treated fruit, all rates were pooled and compared

to untreated fruit. In 1995, the first visible symptoms of internal breakdown were detected at 8 WAFS in 'Tommy Atkins' and 'Van Dyke' fruit (data not shown). At that sampling date, the incidence of internal breakdown was 3 times higher in 'Van Dyke' fruit than in 'Tommy Atkins'. 'Irwin' fruit did not show the disorder until 12 WAFS, and the number of affected fruit remained low throughout fruit ontogeny. However, the ripe 'Irwin' fruit sampled showed no signs of internal breakdown. After the first symptoms of the disorder were noticed in 'Tommy Atkins' and 'Van Dyke', the number of affected fruit increased little from 8 to 16 WAFS. During that 2-month period, there were no differences in the number of affected fruit between 'Tommy Atkins' and 'Van Dyke'. However, when the fruit were ripe, the number of affected fruit increased to four times the number observed at 16 WAFS in 'Tommy Atkins' and 'Van Dyke'.

1995-1996 trial. There were no significant effects of Cab'Y or Packhard concentrations on the incidence of internal breakdown in 'Tommy Atkins' fruit at any time during fruit ontogeny. The number of disordered fruit was similar among treatments. Although the percentages of disordered fruit were higher among untreated fruit, the differences between treated and untreated fruit were not statistically significant. The first signs of internal breakdown were detected 8 WAFS. At that sampling date, 25% of the untreated fruit showed the disorder, whereas 13.9% and 11.1% of the fruit treated with Cab'Y and Packhard, respectively had internal breakdown (data not shown). Jelly seed and SEC were the two first disorders to be detected in the fruit. The first symptoms of soft nose were observed 4 weeks later, i.e. at 12 WAFS, when the fruit were nearly mature. The number of disordered fruit increased with time and reached a maximum of 66% in the untreated group, and 50% and 53% in the fruit treated with Cab'Y and Packhard, respectively, in the ripe fruit.

Table 5-8. Regression equations relating the B concentration (Y) in 'Tommy Atkins' mango fruit with the concentrations of Cab'Y sprayed weekly at 0, 3.6, 5.4, and 7.2 g/L Ca. (** or *= Significant at $P<0.01$ or $P<0.05$; n=12; WAFS = week after fruit set).

WAFS		Regression equations for estimating fruit B concentration			
(1996)	Type of curve	r^2	intercept	slope of x	slope of x^2
4	linear*	0.29	15.19	0.8	
	quadratic**	0.46	18.63	-2.64	0.69
5	linear**	0.54	12.75	1.79	
	quadratic**	0.55	11.34	3.19	-0.28
6	linear*	0.31	15.50	1.10	
	quadratic**	0.49	10.81	5.79	-0.94
7	linear**	0.45	116.35	-27.63	
	quadratic**	0.50	171.46	-82.73	11.02
8	linear**	0.33	14.75	0.95	
	quadratic*	0.36	16.26	-0.56	0.30
9	linear**	0.60	12.96	1.21	
	quadratic**	0.60	12.96	1.21	-0.00
10	linear**	0.76	13.75	1.24	
	quadratic**	0.77	14.32	0.66	0.11
11	linear**	0.56	10.42	1.05	
	quadratic**	0.57	9.74	1.73	-0.14
12	linear**	0.42	8.90	1.24	
	quadratic**	0.47	6.50	3.64	-0.48
14	linear*	0.61	6.25	2.36	
	quadratic**	0.76	12.81	-4.20	1.31

Table 5-9. Regression equations relating B concentration (Y) in 'Tommy Atkins' mango fruit with the concentrations of Packhard sprayed weekly at 0, 3.6, 5.4, and 7.2 g/L Ca. (** or *= Significant at $P<0.01$ or $P<0.05$; n=12; WAFS = week after fruit set).

WAFS (1996)	Type of curve	Regression equations for estimating fruit B concentration			
		r^2	intercept	slope of x	slope of x^2
4	linear**	0.63	12.75	2.69	
	quadratic**	0.84	21.34	-5.91	1.72
5	linear**	0.54	13.25	2.30	
	quadratic**	0.67	7.00	8.55	-1.25
6	linear**	0.58	14.31	2.04	
	quadratic**	0.62	11.34	5.00	-0.59
7	linear**	0.41	98.04	-23.62	
	quadratic**	0.63	195.75	-121.33	19.54
8	linear**	0.44	14.56	1.54	
	quadratic*	0.45	15.50	0.60	0.19
9	linear**	0.82	12.17	2.45	
	quadratic**	0.85	9.72	4.89	-0.49
10	quadratic**	0.75	8.91	7.41	-1.36
11	linear*	0.33	11.46	0.78	
	quadratic**	0.54	7.92	4.33	-0.71
12	linear**	0.59	8.92	1.66	
	quadratic**	0.72	4.59	5.99	-0.86
13	linear**	0.75	7.24	1.78	
	quadratic**	0.75	7.14	1.88	-0.02
14	linear**	0.64	8.09	1.93	
	quadratic**	0.64	8.13	1.88	0.009

Discussion

Foliar $CaCl_2$ sprays did not have a significant effect on the mineral element concentrations in mango fruit. However, the concentrations of several nutrients, especially B, were affected by the Cab'Y and Packhard sprays. The amount of Ca that that actually penetrates into a fruit is influenced by a variety of factors, including timing, rates and frequency of the applications, and environmental conditions (Peryea, 1994). The applied treatments may have been ineffective for several reasons. The deposition of cutin over the fruit exocarp with time is one of the characteristics of mango fruit development (Dietz et al., 1988; Roth, 1977). Glenn et al. (1985) indicated that lenticels, cuticle cracks, and stomata are important in the pathway of Ca diffusion through the cuticle of the apple fruit. In this study with mango, experiments began 4 WAFS in both 1995 and 1996. At this stage of fruit ontogeny, the cuticle layer may have already been formed on the fruit epidermis, preventing the penetration of the applied chemicals. The thickness of the wax layer in papaya fruit increases from 15 to 50 μm during fruit ontogeny (Quintana and Paull, 1993). The scientific literature does not contain similar details for the cultivars used in this study. However, Dietz et al. reported increasing cuticular thickness throughout fruit ontogeny in five mango cultivars (Dietz et al., 1988). There is a possibility that the $CaCl_2$, Cab'Y and Packhard treatments were ineffective due to inherent anatomical barriers in the mango fruit rather than to the lack of efficacy of the chemicals. Moreover, Cab'Y and Packhard were more effective in affecting the concentrations of several mineral elements, including Ca, at the earliest sampling date and when the fruit were ripe. It is possible that these two stages of fruit ontogeny are the most suitable for penetration by exogenously applied chemicals. The cuticle layer may be thinner in the youngest fruit, whereas physical changes, such as cuticle cracking, that probably took place in the fruit exocarp

during fruit ripening may predispose the ripe fruit to similar penetration by the applied chemicals.

Differences in the formulations of the chemicals may also have contributed to the results reported in this study. Calcium chloride alone was applied in 1995, whereas Cab'Y and Packhard, which contain B, were applied in 1996. Boron is thought to improve the solubilization of certain minerals, especially Ca, in plant tissues (Odet and Dumoulin, 1993; Zekri, 1995). Thus, the observed relationship between the concentrations of Cab'Y or Packhard and the fruit concentrations of certain mineral elements in the fruit may be related to the presence of B. However, the B concentration may have not been sufficient to maximize Ca solubility.

Calcium deficiency has been shown to negatively affect the quality of many fruit and vegetables. Raese (1994) studied the relationship of fruit size and crop load to fruit Ca and the incidence of cork spot in 'd'Anjou' pears for 13 years. The incidence of the disorder varied over time and occurred at a higher percentage in fruit with a low Ca concentration. For example, 36.1% of fruit were affected with cork spot in 1982 as opposed to 5.5% in 1985. Average fruit Ca was 2.25 mg 100 g^{-1} in 1982 compared to 2.68 mg 100 g^{-1} in 1985. Curtis et al. (1990) studied the concentrations of N, Ca, P, and Mg in healthy and cork-spotted 'd'Anjou' pears for 2 years. The incidence of cork-spot was higher when the N:Ca ratio of the peel was at least 6.3. Curtis et al. (1990) suggested that the peel N:Ca ratio was a better index to predict the occurrence of the disorder than fruit Ca concentration alone. In a study of the chemical composition of healthy and disordered 'Alphonso' mangoes collected from eight locations in India, Subramanyan et al. (1971) observed that fruit affected with spongy tissue had lower Ca (74 mg 100 g^{-1}) and higher P (120 mg 100 g^{-1}) concentrations than normal fruit (85 mg 100 g^{-1} Ca and 96 mg 100 g^{-1} P, respectively)

Calcium chloride has been successfully used to control other Ca-related fruit disorders. Evans and Troxler (1968) observed that tomatoes sprayed weekly with 10

g/L $CaCl_2$ produced fewer fruit with blossom-end rot (26%) than plants sprayed with water (56%) or non-sprayed plants (38%). The combination of a $CaCl_2$ spray plus a Ca gluconate injection completely prevented the disorder and resulted in significantly higher fruit Ca concentrations. Beyers (1963) observed that pre-harvest foliar applications of 10 g/L $CaCl_2$, 6 g/L $Ca(NO_3)_2$, and 20 g/L $Ca(NO_3)_2$-$CO(NH_2)_2$ were equally effective in reducing the incidence of bitter pit in apple, whereas the disorder occurred with higher incidence in trees that were treated with Ca acetate. In a similar experiment, Raese (1994) observed that bitter pit in 'Delicious' and 'Golden Delicious' apples was effectively controlled by $CaCl_2$ and $Ca(NO_3)_2$, whereas $CaSO_4$ or Chelazone (an amino acid-Ca chelated material) were ineffective.

Conway and Sams (1987) observed that pressure infiltrating 'Delicious' apples with 80 g/L $CaCl_2$ reduced the decay area by 46% compared to 14% and 18% obtained with comparable concentrations of $MgCl_2$ or $SrCl_2$, respectively. Fruit treated with 80 g/L $CaCl_2$ were 25% firmer than the control, and 17% and 14% firmer than fruit treated with comparable solutions of $MgCl_2$ and $SrCl_2$, respectively.

The application of Ca as $CaCl_2$, Cab'Y or Packhard did not significantly reduce the incidence of internal breakdown in mango fruit. Concurrent studies were conducted with $CaCl_2$ sprays in 1995, and with Cab'Y and Packhard dips in 1996 to solely determine the effects of these treatments on internal breakdown in tree-ripened mango fruit (F. Sesto, Brooks Tropicals Inc., pers. comm., 1997). In those studies, fruit were not sampled for nutrient determination. Thus, all fruit remained on the trees allowing for a large sample size of tree-ripened fruit. In both years, there were no differences in the percentages of disordered fruit among the CaCl2, Cab'y, or Packhard treatments (F. Sesto, Brooks Tropicals Inc., pers. comm., 1997).

There have also been reports of $CaCl_2$ being ineffective in controlling or preventing Ca-related fruit disorders in other crop species. Facteau et al. (1987) observed no reduction of surface pitting or bruising of 'Bing' and 'Lambert' sweet

cherry (*Prunus avium* L.) fruit that were given preharvest sprays of $CaCl_2$ (950 mg/L, 1400 mg/L, and 3800 mg/L Ca in single or multiple applications). However, in three out of the seven trials, fruit firmness and fruit size increased as the amount of applied Ca increased. Krishnamurthy (1982) observed no differences in the percentages of internal breakdown in 'Alphonso' mango fruit treated with 5g/L $CaCl_2$ alone or in combination with 0.5 g/L B applied as pre-harvest foliar sprays or post-harvest dips. Similarly, Qiu et al. (1995) observed in papaya fruit that biweekly applications of 2 g/L $CaCl_2$, or post-harvest infiltration and dipping of the cut peduncle into $CaCl_2$ solutions failed to increase the concentration of Ca in the mesocarp.

The intensity of the disorder varied among the cultivars. 'Van Dyke' showed more susceptibility to the disorder than 'Tommy Atkins' or 'Irwin'; 'Irwin' was the least susceptible and 'Tommy Atkins' was intermediate. This observation is supported by a previous report made by Galán-Sauco et al. (1984) who studied the influence of harvest stage and harvesting date on the incidence on internal breakdown in 26 mango cultivars. It was observed that early harvesting reduced the incidence of the disorder and that the disorder was cultivar related (Galán-Sauco et al., 1984). In a later report, Mead and Winston (1991) confirmed that assertion. The results presently reported (Table 5-1) indicate that 'Irwin' is, to a lesser extent than 'Tommy Atkins' and 'Van Dyke', also susceptible to internal breakdown. This contrasts with the prevailing belief among south Florida mango growers who tend to consider 'Irwin' as a tolerant cultivar to internal breakdown. However, this observation is in agreement with Malo and Campbell's report (1978) in which they indicated that 'Irwin' was among the cultivars susceptible to this disorder.

Internal breakdown of mango fruit is considered to be a cultivar-related disorder (Galán-Sauco et al., 1984; Malo and Campbell, 1978; Mead and Winston, 1991). The results from the 1994-1995 study support this view, and indicate that the incidence of internal breakdown differed among cultivars not only by its severity but also in the time

that the symptoms first appeared. For example, in 1995, 'Irwin' fruit first showed the disorder at 12 WAFS, whereas the first symptoms were detected in 'Tommy Atkins' and 'Van Dyke' at 8 WAFS. In 'Irwin', the number of disordered fruit never exceeded 22% of the fruit sampled, even in the control treatment. During that same year, a maximum of 47% of the treated 'Van Dyke' fruit and 53% of the 'Tommy Atkins' fruit were affected.

Although the maximum incidence of internal breakdown was observed mainly during the latest stages of fruit ontogeny, symptoms could be detected earlier in fruit development. Thus, internal breakdown is not exclusively restricted to mature or ripe mango fruit. It is probable that the disorder is induced earlier than the moment at which symptoms are first observed. In this sense, the disorder may be viewed as a cause rather than a result of unbalanced ripening in the mango fruit.

Under the conditions of these experiments, it was not possible to determine if Ca concentrations in the fruit were related to internal breakdown in mango. There is a need to conduct further studies that target earlier stages of fruit development, and eventually the blooming period, to increase the chance of absorption of the chemicals into the fruit. Differences in the fertilization programs, which differed among orchards, may also have influenced the mineral element concentrations in the mango fruit.

Conclusions

Under the conditions of these experiments, $CaCl_2$ sprays applied in 1995 were not effective in increasing fruit Ca concentration or in reducing internal breakdown in mango. Concentrations of Cab'Y or Packhard applied as foliar sprays in 1996 were related to the concentrations of various nutrients, particularly N, K, Ca, Zn, Mn, Cu, and B, in the fruit. There was a strong relationship between Cab'Y and Packhard application rates and fruit B concentrations. Thus, these two products may represent good sources for supplying B in cases of deficiencies in fruits. Young fruit appeared to

be more responsive to Cab'Y or Packhard sprays than older fruit. In general, Packhard was more effective than Cab'Y in increasing mineral element concentrations in the fruit. Observations from the 1995-1996 trial revealed that the concentrations of some mineral elements in the ripe fruit were related to the application rates of Cab'Y or Packhard, but this may not be an appropriate time for applying exogenous Ca, as the disorder is already in its advanced stages when fruit are ripe. Thus, chemical applications may have been scheduled too late during fruit development. Cuticle formation may have contributed to preventing effective penetration of Ca into the fruit mesocarp. Although there is no evidence that $CaCl_2$, Cab'Y, or Packhard would have an effect on internal breakdown if they were effectively absorbed, it is possible that starting Ca applications at earlier stages of fruit ontogeny may improve the chances of increasing the Ca concentrations in the mesocarp of the fruit, consequently reducing the incidence of the disorder. Differences in cultural practices among orchards may have interfered with the treatments. In addition, rains that occurred during the experiments may also have reduced the effectiveness of the treatments by washing away the applied materials. The results obtained from these experiments do not support or refute the hypothesis that Ca deficiency is the primary cause of internal breakdown in mango fruit. Further investigations with more controlled conditions are needed to address this issue.

CHAPTER VI

COMPARISON OF FRUIT MINERAL ELEMENT CONCENTRATIONS AND FRUIT WEIGHT BETWEEN MANGO FRUIT WITH AND WITHOUT INTERNAL BREAKDOWN

Introduction

The fruit of several commercial mango cultivars are affected by various physiological disorders such as black tip (Agarwala et al., 1962; Srivastava, 1963; Zhang et al., 1995), internal necrosis (Ram, 1988; Ram et al., 1988), fruit splitting (Lim and Koo, 1985), and internal breakdown (Subramanayam et al., 1971; Verma, 1950; Young, 1957). Internal breakdown is thought to prevail in Indian cultivars or cultivars with an Indian pedigree (Schaffer, 1994; Young, 1957). Depending upon the symptoms and growing regions, internal breakdown is referred to as 'jelly seed', 'soft nose', 'stem-end cavity' (SEC), 'yeasty fruit rot', 'insidious fruit rot', or 'flesh breakdown'. The disorder has been related to several variables, including environmental factors (Lad et al., 1992), cultural practices (Malo and Campbell, 1978), a nutritional imbalance or deficiency (Burdon et al., 1991), fruit weight (Subramanyam et al., 1971) and fruit specific gravity (Krishnamurthy, 1980).

There is much controversy surrounding the nature or cause of internal breakdown in mango fruit. In studies conducted in Florida, increased N fertilization increased the incidence of soft nose (Young, 1957; Young and Miner, 1961; Young et al., 1962). However, in another study in Florida, N fertilization or high leaf N concentrations could not be correlated with the incidence of internal breakdown (Malo and Campbell, 1978). Increased irrigation (Malo and Campbell, 1978) or rain

(Katrodia, 1988) at the time of fruit development have also been considered to increase the occurrence of internal breakdown in mango. However over a six-year period, no differences in the percentages of disordered 'Sensation' fruit were observed among irrigation treatments that maintained soil matric potentials at -20, -50, or -70 kPa (Farre and Hermoso, 1993).

Calcium deficiency is considered to be the most probable cause of internal breakdown (Shear, 1975; Young, 1957). However, there is no definitive evidence for the role of Ca in the development of the disorder. In a study of the distribution of P, K, Ca, and Mg in mango fruit susceptible to the soft nose disorder, Burdon et al. (1991) observed that the Ca and Mg concentrations in disordered 'Kent' fruit were lower than those in the healthy 'Kent' fruit. It was also observed that Ca and Mg concentrations were equally low in healthy and disordered 'Beverly' mango fruit. However, the disordered mesocarp of 'Beverly' mangoes contained significantly higher Ca, Mg, K, and P concentrations than the healthy mesocarp (Burdon et al., 1991). Krishnamurthy (1981) found no relationship between internal breakdown and Ca concentrations in the mesocarp of 'Alphonso' mango fruit. Gunjate et al. (1979) reported a reduced incidence of spongy tissue in 'Alphonso' fruit following preharvest or postharvest dips of the fruit in 5 or 20 g/L $CaCl_2$ or $Ca(NO_3)_2$. However, pre-harvest sprays of 5 g/L $CaCl_2$ alone or in combination with 0.5 g/L H_3BO_4 did not reduce the incidence of internal breakdown, and had no effect on the fruit Ca level (Krishnamurthy, 1982). In that study, the post-harvest dips of the fruit into 2.5 or 5 g/L $CaCl_2$ solution baths for 5 minutes also failed to reduce the incidence of internal breakdown (Krishnamurthy, 1982). In addition, Gautam and Lizada (1984) (as cited by Burdon et al., 1991; Lad et al., 1992; Wainright and Burbage, 1989), observed higher Ca concentrations in disordered 'Carabao' fruit than in healthy fruit.

Comparing the concentrations of specific nutrients in healthy fruit with those in disordered fruit would help to elucidate the role of mineral nutrition in internal

breakdown. The purpose of this study was to compare the concentrations of N, P, K, Ca, Mg, Zn, Cu, Mn, Fe, and B in the fruit, and fruit weight between healthy and disordered mango fruit, and to determine if there is a relationship between these fruit mineral element concentrations and the occurrence of internal breakdown in 'Tommy Atkins' fruit throughout fruit development.

Materials and Methods

Location. The study was conducted during the 1996 fruiting season in a commercial orchard in south Dade County (25.36°N and 80.21°W). The orchard was established on a Krome soil (loamy, skeletal, carbonatic, hyperthermic Lithic Udorthents). These soils are 0-27.50 cm deep and made of very gravelly loam or weathered bedrock. They contain 15-20% clay and have a pH of 7.4-8.4 (USDA, 1996). Daytime temperatures during the experimental period averaged 23.6°C and the total rainfall amount was 894.5 mm, with a peak in June.

Plant materials. Eight 30-year-old trees of 'Tommy Atkins' grafted on 'Turpentine' rootstock were used in the study. No fertilizer was applied during the experiment. However, the trees had received 568 kg/ha of 6-0-19 (N-P-K) in March 1995, 9.94 kg/ha of Sequestrene-Fe (Geigy 138, 5% Fe) in August 1995, 852.27 kg/ha of 3-8-12 and 19.32 kg/ha of Sequestrene-Fe in September 1995, and 19.31 kg/ha of Sequestrene-Zn (Geigy 138, 14% Zn) plus 19.32 kg/ha of Sequestrene-Mn (Geigy 138, 12% Mn) in December 1995.

Sampling. Three fruit were collected every week from each tree, beginning four weeks after fruit set (WAFS) until the fruit were ripe. There were four two-tree replicates. The fruit were considered to be ripe when the mesocarp was sufficiently soft to allow consumption as a fresh fruit. The samples were placed in paper bags and

shaded to prevent water loss that could result from prolonged exposure to the sun during sampling and transportation from the field to the laboratory.

Determination of fruit weight and internal breakdown. Fruit weight was determined by individually weighing the fruit immediately after collection from the orchard. The presence of internal breakdown was determined after cutting open the fruit samples. Each fruit was first transversally cut at the proximal end, between the peduncle and the base of the stone. The objective of the transverse cut was to assess the presence of stem-end cavity in the fruit. Two additional longitudinal cuts were made on each of the wider flat sides of the stone to expose the interior of the fruit and detect the presence of either jelly seed or soft nose.

Processing of samples. Collected fruit were washed in a 10 ml/L detergent solution, rinsed in tap water, washed in 0.6 M HCl, and rinsed twice in distilled water as described by Schaffer et al. (1988). After washing, the fruit were weighed, and cut open, as previously described, and oven dried for 48 to 120 hours, depending upon the size of the fruit. Dried samples were ground in a cyclone mill (UDY Corp., Fort Collins, CO).

For P, K, Ca, Mg, Zn, Cu, Mn, Fe, and B determination, 1 g of ground tissue was weighed in a 40-ml high-form porcelain crucible (Fischer Scientific, Pittsburgh, PA) and ashed at 500°C in a muffle furnace (Furnatrol FA 1730, Barnstead/Thermolyne, Dubuque, IA). The ashed sample was digested with 5 ml of 6 M HCl and brought to 50 ml with deionized water in a polyethylene volumetric flask. The preparation was shaken and filtered through Whatman #1 filter paper into a 20-ml scintillation vial. Polyethylene vials were used instead of glass so that there was no borosilicate to interfere with B determinations.

For N determination, 0.2 g of ground tissue was weighed into a 100-ml digestion tube to which 2 g of Kjeldahl mixture and 5 ml H_2SO_4 were added. Glass funnels were placed on the tubes, and the tubes and funnels were placed on a preheated

aluminum digestion block at 250°C for 1 hour. The temperature of the digestion block was raised to 380°C for an additional 3-hour period. After the tubes cooled to room temperature, 5 ml of distilled water were added to each tube and the preparation was agitated with a vortex mixer. The digested material was transferred to a 100-ml volumetric flask, and the content was vigorously mixed and filtered through Whatman #1 filter paper into a 20-ml polyethyelene scintillation vial (Hanlon et al., 1994). Nitrogen concentrations were determined by the Total Kjeldahl Nitrogen (TKN) method and K, P, Ca, Mg, Zn, Cu, Mn, Fe, and B were determined by inductively coupled argon plasma spectroscopy (ICAP).

Results

Fruit fresh weight. Significant differences in fruit fresh weight existed between healthy and disordered fruit throughout most of the fruit development period (Fig. 6A). However, no definite pattern was observed in the relationship between fruit weight and internal breakdown. Healthy fruit weighed significantly less than disordered fruit when symptoms of internal breakdown were first detected 8 WAFS, but when the fruit were ripe, the healthy fruit were heavier than the disordered fruit. Healthy fruit weighed more than disordered fruit 9 and 12 WAFS. However, the opposite was observed 11 and 13 WAFS. There were no differences in fruit fresh weight between healthy and disordered fruit 10 WAFS.

Development of internal breakdown symptoms. The time at which the first signs of internal breakdown appeared varied with the cultivars and the types of disorder. In 1995, 'Tommy Atkins' and 'Van Dyke' fruit were affected by SEC and jelly seed as early as eight WAFS. At that sampling date, the 'Tommy Atkins' fruit were approximately 17% of their full weight, and fruit of 'Van Dyke' 10%. The first symptoms of SEC and jelly seed appeared in 'Irwin' fruit 12 WAFS, when the fruit had

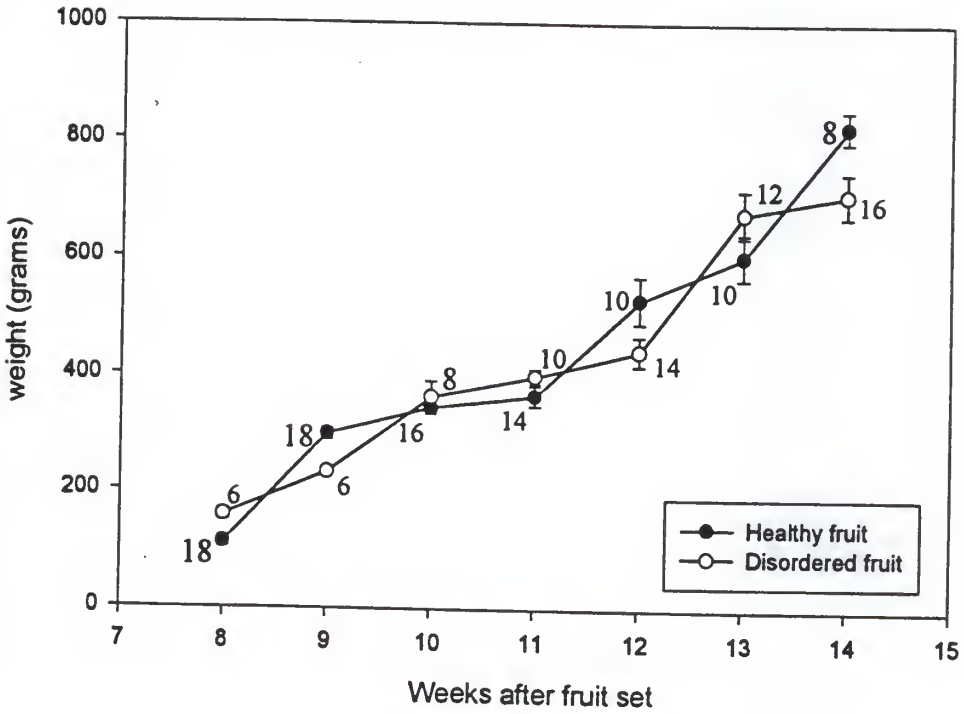


Fig. 6-1. Fresh weight of healthy and disordered 'Tommy Atkins' mangoes from first signs of internal breakdown to fruit ripeness. Values on the left or right of symbols represent the numbers of healthy or disordered fruit respectively. Vertical bars represent ± 1 standard error. Absence of error bars indicates that the standard error was smaller than the symbol for the mean.

attained 68% of their final weight. In 1996, SEC and jelly seed were the first disorders detected in the 'Tommy Atkins' fruit 8 WAFS. During both years, symptoms of the soft nose disorder appeared only when the fruit were nearly mature. For the purpose of this study, although fruit sampling commenced 4 WAFS, the data presented for fruit mineral concentrations were selected so that they correspond to the time at which internal symptoms were noticed.

Mineral elements. The concentrations of N were higher in disordered fruit than in healthy fruit at 8 and 9 WAFS, i.e. during the two weeks after the first signs of internal breakdown were detected (Fig. 6-2A). There were no differences in fruit N concentrations between disordered and healthy fruit at 10 WAFS and 12 WAFS, and when the fruit were ripe. Nitrogen concentrations fluctuated more in the disordered fruit than in the healthy fruit. For example, N concentrations in healthy fruit were fairly consistent from 10 to 13 WAFS, whereas significant fluctuations in fruit N concentrations occurred in disordered fruit.

The P concentrations of healthy fruit were significantly lower than those of disordered fruit at 8 and 9 WAFS, i.e. during the first two weeks after symptoms of internal breakdown appeared (Fig. 6-2B). Phosphorus concentrations in healthy fruit were nearly equal to those in disordered fruit during the remaining period of fruit development, except at 11 WAFS, when P concentrations were higher in the healthy fruit than in the disordered fruit. The P concentrations of disordered fruit did not show any significant changes from 10 to 13 WAFS, whereas a significant fluctuation occurred in the P concentration in healthy fruit at 11 WAFS. There was a significant increase in the P concentrations in the disordered fruit when those fruit were ripe, i.e. at 14 WAFS.

The concentrations of K in healthy and disordered fruit fluctuated throughout fruit development. Fruit K concentrations in the healthy fruit were not significantly different from those observed in the disordered fruit at 8 and 10 WAFS, and when the

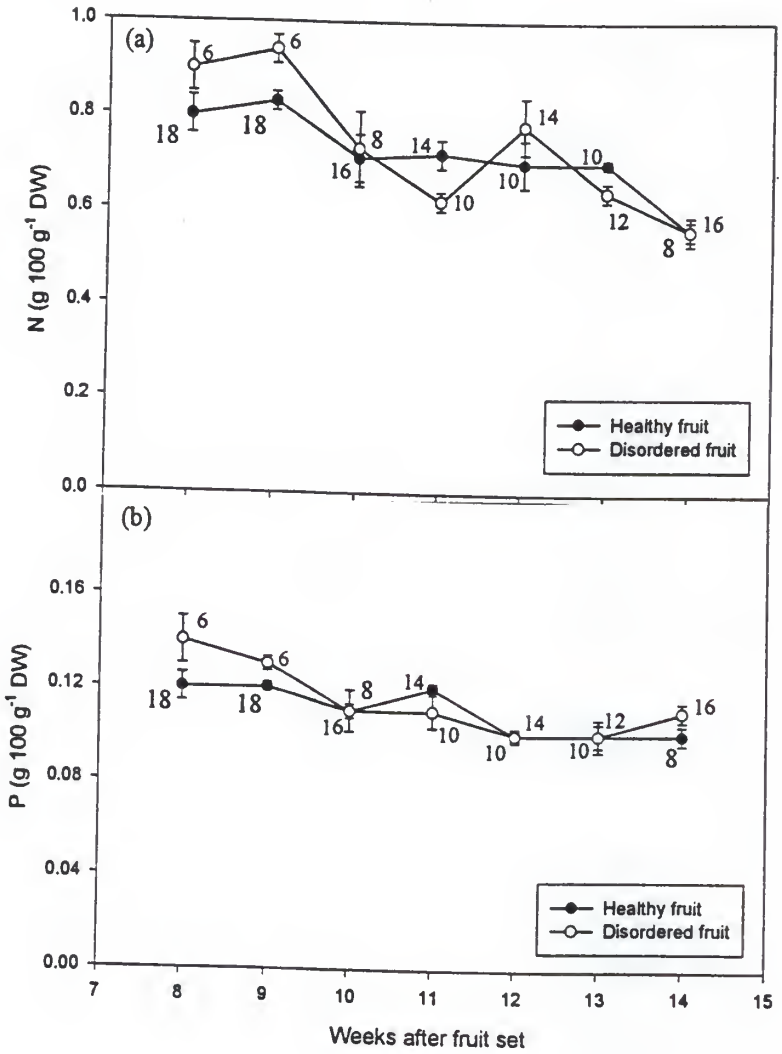


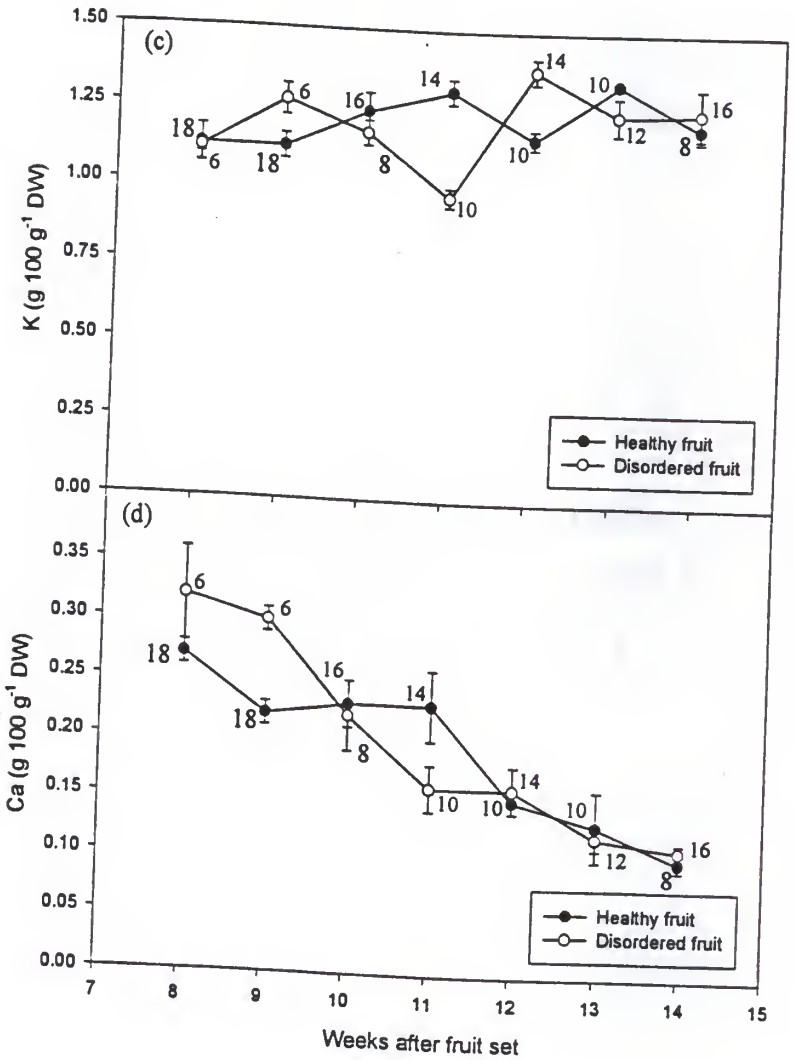
Fig. 6-2. Concentrations of N, P, K, Ca, Mg, Zn Cu, Mn, Fe, and B in healthy and disordered 'Tommy Atkins' mangoes from first signs of internal breakdown to fruit ripeness. Values on the left or right of symbols represent the numbers of healthy and disordered fruit, respectively. Vertical bars represent ± 1 standard error. Absence of error bars indicated that the standard error was smaller than the symbol for the mean.

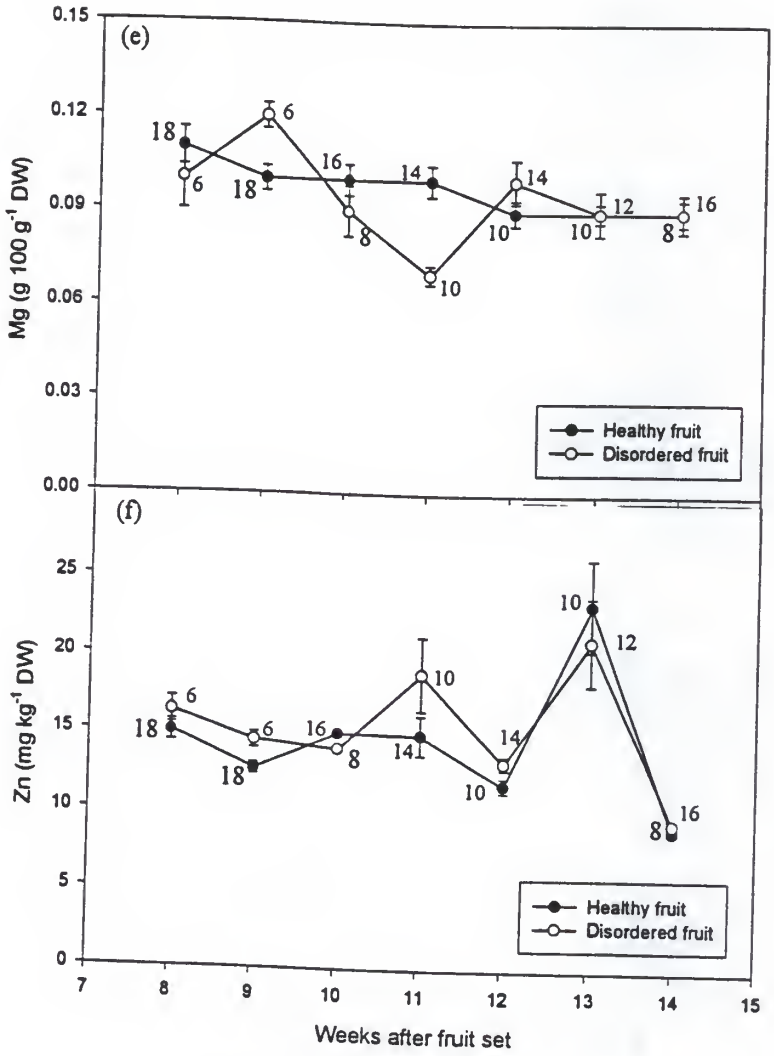
fruit were ripe (Fig. 6-2C). The fruit K concentrations in healthy fruit were higher than that in disordered fruit at 11 and 13 WAFS, whereas disordered fruit contained higher fruit K concentrations than healthy fruit at 9 and 12 WAFS.

Concentrations of Ca in healthy fruit were similar to those in disordered fruit throughout the sampling period. No differences in the fruit Ca concentrations were observed between healthy and disordered fruit 8 WAFS, i.e. when the symptoms of internal breakdown were first detected, although variability in Ca concentrations among the disordered fruit was much greater (Fig. 6-2D). Calcium concentration in the disordered fruit was significantly higher than the concentration observed in the healthy fruit 9 WAFS, whereas healthy fruit contained significantly higher Ca concentrations than disordered fruit 11 WAFS. From 12 WAFS until the fruit were ripe, there were no differences in fruit Ca concentrations between healthy and disordered fruit.

There were no differences in Mg concentrations between healthy and disordered fruit when the symptoms of internal breakdown were first detected 8 WAFS. The Mg concentration of the disordered fruit significantly increased between 8 and 9 WAFS, whereas that of the healthy fruit significantly declined such that healthy fruit had significantly lower fruit Mg concentrations than disordered fruit 9 WAFS (Fig. 6-2E). The increase in Mg concentration in the disordered fruit observed between 8 and 9 WAFS was followed by significant declines 10 and 11 WAFS. In the healthy fruit, no significant changes in Mg concentrations were observed from 9 to 11 WAFS. Another increase in Mg concentration of the disordered fruit was observed between 11 and 12 WAFS, whereas the Mg concentrations of the healthy fruit significantly declined during that period. There were no differences in Mg concentrations between healthy and disordered fruit from 12 WAFS until the fruit were ripe.

Concentrations of Zn significantly fluctuated with time in healthy and disordered fruit. When the symptoms of internal breakdown were first noticed 8 WAFS, there were no significant differences in Zn concentrations between healthy and


(c)
K (g 100 g-1 DW)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Healthy fruit
Disordered fruit
(d)
Ca (g 100 g-1 DW)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
7
8
9
10
11
12
13
14
15
Weeks after fruit set


(e)
Mg (g 100 g-1 DW)
0.15
0.12
0.09
0.06
0.03
0.00
Healthy fruit
Disordered fruit
(f)
Zn (mg kg-1 DW)
25
20
15
10
5
0
7
8
9
10
11
12
13
14
15
Weeks after fruit set
Healthy fruit
Disordered fruit

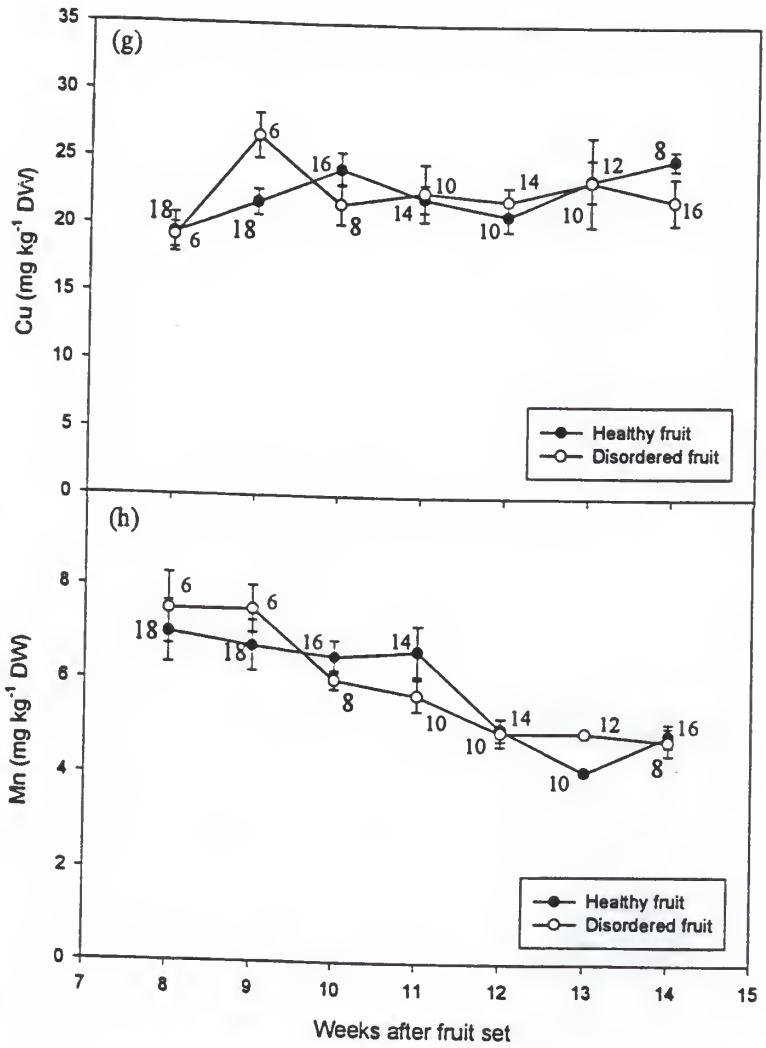
disordered fruit (Fig. 6-2F). Healthy and disordered fruit also contained similar concentrations of Zn at 13 WAFS and when the fruit were ripe. Healthy fruit had significantly lower Zn concentrations than disordered fruit at 9, 11, and 12 WAFS, whereas Zn concentrations were lower in the disordered fruit at 10 WAFS. Zinc concentrations fluctuated widely from 10 to 14 WAFS in disordered fruit and from 11 to 14 WAFS in healthy fruit, fluctuating $\pm$ 30 to 60% from the concentrations observed at 10 WAFS

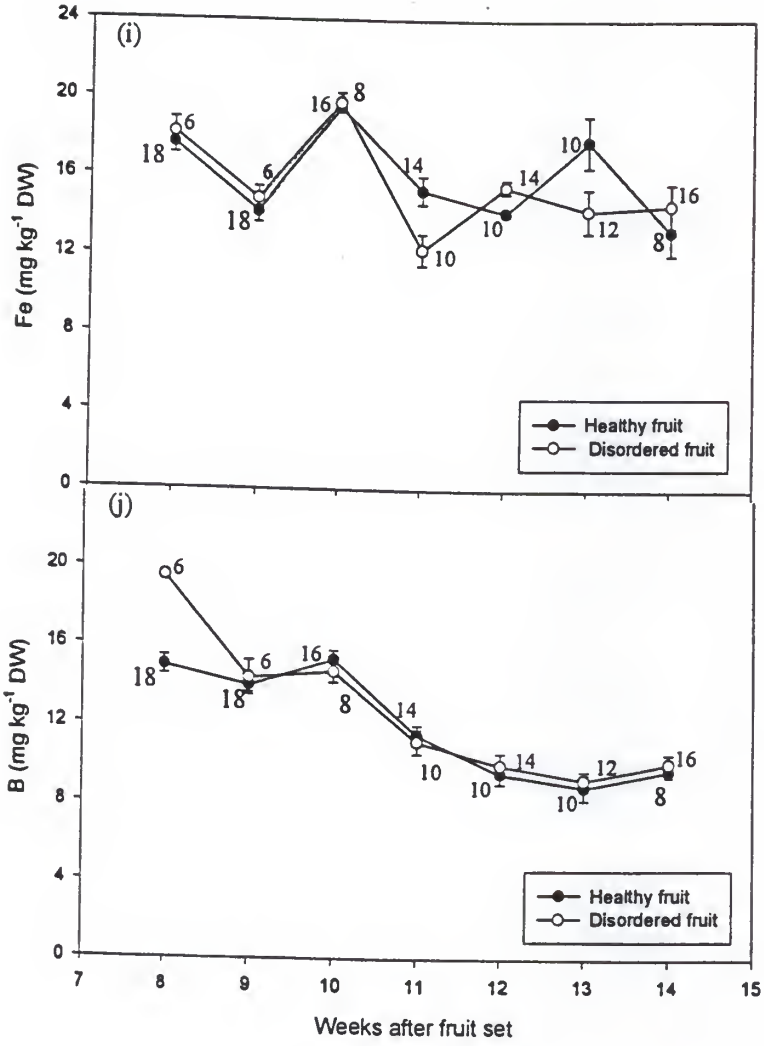
Throughout the sampling period, Cu concentrations remained fairly similar in healthy and disordered fruit. However, Cu concentrations were significantly higher in healthy fruit than in disordered fruit when the fruit were ripe, whereas disordered fruit had higher Cu concentrations than healthy fruit at 9 WAFS (Fig. 6-2G).

There were no significant differences in Mn concentrations between healthy and disordered fruit throughout most of the fruit development period (Fig. 6-2H). The Mn concentrations of healthy fruit did not change significantly from 8 to 11 WAFS. During the same period, the Mn concentration in the disordered fruit had declined 20% by 10 WAFS. The Mn concentration in the healthy fruit was significantly higher than that of the disordered fruit at 11 WAFS, whereas the reverse was observed at 13 WAFS. When the fruit were ripe, no differences in Mn concentrations were observed between healthy and disordered fruit.

The Fe concentrations in healthy fruit were similar to those in disordered fruit from 8 to 10 WAFS and when the fruit were ripe (Fig. 6-2I). Iron concentrations were higher in healthy fruit at 11 and 13 WAFS, whereas disordered fruit contained slightly higher Fe concentrations at 12 WAFS.

Healthy fruit had similar B concentrations as disordered fruit throughout most of the fruit development period, except at 8 WAFS, at which time B concentration was significantly higher in the disordered fruit than in the healthy fruit (Fig. 6-2J).


(g)
Cu (mg kg-1 DW)
Healthy fruit
Disordered fruit
(h)
Mn (mg kg-1 DW)
Healthy fruit
Disordered fruit
Weeks after fruit set


(i)
Fe (mg kg-1 DW)
Healthy fruit
Disordered fruit
(j)
B (mg kg-1 DW)
Healthy fruit
Disordered fruit
Weeks after fruit set

Discussion

No relationship existed between fruit weight and the occurrence of internal breakdown. Subramanyam et al. (1971) reported an increased incidence of internal breakdown in 'Alphonso' mangoes with increasing fruit weight. In that study, the fruit were harvested at the mature-green stage and allowed to ripen in ventilated wooden boxes at 28 ± 3°C and 60-90% relative humidity. When the fruit were ripe, the percentages of disordered fruit were 18.2%, 25.4%, 36.4%, and 44.5% for weight classes of <200 g, 200-250 g, 250-300 g, and >300 g, respectively. There are large differences in weight between 'Tommy Atkins' and 'Alphonso' mangoes. 'Tommy Atkins' fruit weigh approximately 500 g at maturity (Fig. 21). It is possible that the lack of correlation between the fruit fresh weight and the incidence of internal breakdown was due to the fact that the 'Tommy Atkins' fruit exceeded the weight range reported in that study.

The incidence of internal breakdown in mango may not be the result of a nutrient deficiency since there were few differences in the concentrations of fruit mineral elements between healthy and disordered fruit. A number of studies have related high N concentrations to the incidence of physiological disorders in mango. Young (1957) observed an increasing incidence of soft nose with increased N fertilization of 'Kent' mango trees. In that study, the percentages of fruit with definite symptoms of soft nose were 7.7%, 9.6%, and 11.9% harvested from trees that received 90, 180, or 360 g of N, respectively. It was also observed that the severity of the disorder was higher for trees in acidic, sandy soils than for trees in calcareous soils. A study of the effects of N and K fertilization on the incidence of internal breakdown in 'Tommy Atkins' fruit failed to produce conclusive evidence for a relationship between high N concentrations in the leaves and internal breakdown (Malo and Campbell, 1978). In that 5-year study, the treatments were 0.68, 1.36, and 2.72 kg of K/tree/year, and 0.34, 0.68, and 1.36 kg of N/tree/year (Malo and Campbell, 1978).

In these previous studies, mineral element concentrations were only determined in leaves and there were no determinations of fruit mineral element concentrations. Results presented in a previous chapter indicated large differences in the concentrations of mineral elements between mango fruit and leaves (ref. Chapter IV). Therefore, leaf nutrient concentrations may not be the best indicators of the relationship between the nutritional status of the tree and internal breakdown of the fruit.

Similar to the results observed in this study, Joshi and Limaye (1984), as cited by Lad et al. (1992), reported that various application rates of N, P, and K did not have a significant effect on the occurrence of spongy tissue in 'Alphonso' mango, but N concentration in the fruit before ripening was not reported. The relationship between leaf N and internal breakdown has raised some controversy (Young, 1957; Young and Miner, 1961; Malo and Campbell, 1978). There is almost no information on the relationship between N concentration in mango fruit throughout fruit development and the incidence of the disorder. During the accelerated growth phase of mango fruit, cell enlargement takes place. During this period, excessive N in the fruit mesocarp may accentuate the cell enlargement process and, consequently, contribute to weakening of the structural arrangement of the cell wall polysaccharides. Higher N concentrations in the disordered fruit may also have resulted from *de novo* synthesis of enzymes such as the hydrolases, α-amylase, and cellulases. An increase in the activity of these enzymes results in the breakdown of the starch molecules, cell wall softening, and also cell wall deterioration. Other enzymes such as ACC synthase, ACC oxydase, malic enzyme, and pectin methylesterase may also be involved. A study of the chemical composition of 'Alphonso' fruit indicated the activities of malic enzyme (7.2 μmol min^{-1} mg^{-1} protein) and pectin methylesterase (1.60 μeq. min^{-1} mg^{-1} protein) in disordered fruit were significantly higher than the activity levels (4.6μmol min^{-1} mg^{-1} protein and 0.97 μeq. min^{-1} mg^{-1} protein, respectively) of the enzymes in healthy fruit (Krishnamurthy, 1981).

No differences in Ca concentrations were found between disordered and healthy fruit at the beginning and at the end of the sampling period, i.e. when the fruit were youngest and when the fruit were ripe. These results are in agreement with observations made by Burdon et al. (1991) who observed no differences in Ca concentrations between disordered and healthy fruit of 'Beverly' trees at the same site, whereas significant differences in the percentages of disordered fruit existed between 'Kent' and 'Beverly' (Burdon et al., 1991). Similarly, Krishnamurthy (1981) was unable to correlate internal breakdown in 'Alphonso' mango with Ca deficiency in the fruit. The disordered mesocarp of 'Beverly' fruit had higher Ca concentrations than healthy mesocarp (Burdon et al., 1991). In that study, the fruit mesocarp was divided into exterior and interior portions of the mesocarp, and apical, mid, and stem-end sections. In the present study, fruit tissues were not separated, i.e. each fruit was considered as a whole. Consequently, the differences between the results reported in this chapter and those reported by Burdon et al. (1991) may be related to fruit sectioning procedures. Internal breakdown may be the result of redistribution or compartmentalization of nutrients within the fruit (Burdon et al., 1991).

Leaf Ca concentrations have been considered to be a valuable criterion for predicting internal breakdown in mango (Malo and Campbell, 1978; Young, 1957; Young and Miner, 1961). Young (1957) and Young and Miner (1961) indicated that soft nose in 'Kent' was correlated with low foliar Ca concentrations, whereas Malo and Campbell (1978) did not find any relationship between leaf Ca concentrations and internal breakdown in 'Tommy Atkins' mangoes. However, the use of foliar analysis for predicting fruit disorders may not be the best criterion. Calcium primarily moves in the transpiration stream in the xylem (Kirkby and Pilbeam, 1984; Mengel and Kirkby, 1982), which is well developed in leaves. However, the xylem is reduced in the mesocarp of most fruit, and the vascular system mainly consists of phloem (Esau, 1977), in which Ca moves slowly. These anatomical differences predispose mango fruit

to limited Ca absorption compared to the leaf. Thus, in mango, leaves are probably stronger sinks for Ca than fruit. Therefore, the fruit nutritional status should be a better criterion to use for diagnostic purposes related to internal breakdown. Internal breakdown does not affect mango varieties with fibrous fruit (Malo and Campbell, 1978). This may be related to a more efficient vascular network in fibrous fruit. In addition to internal breakdown, Ca deficiency in the fruit has been associated with several other mango fruit disorders. Agarwala et al. (1962) observed that Ca concentrations in healthy 'Safeda' and Tamboori' fruit were more than twice those observed in fruit affected by the black tip disorder. Subramanyam (1971) also observed higher Ca concentrations in the healthy tissues (85 mg 100 g^{-1}) compared to disordered tissues (74 mg 100 g^{-1}) of 'Alphonso' fruit affected by internal breakdown. Gunjate et al. (1979) observed that dipping mango fruit in 5 or 20 g/L $CaCl_2$ or $Ca(NO_3)_2$ significantly reduced the incidence of spongy tissue and that treated fruit contained appreciably higher Ca concentrations than untreated fruit. However, Krishnamurthy (1982) did not observe any reduction of spongy tissue or an increase in the Ca concentrations when 'Alphonso' fruit were treated with 5 g/L $CaCl_2$ in pre-harvest sprays, or with 5 g/L $CaCl_2$ alone or in combination with 0.5 g/L H_3BO_4 in post-harvest dips.

Disordered fruit contained higher P concentrations than healthy fruit when the disorder was first detected and when the fruit were ripe. These results are in agreement with observations previously reported by several researchers. Agarwala et al. (1962) observed that black tip-disordered tissues of 'Safeda' and 'Tamboori' fruit contained higher P concentrations than the healthy tissues, and that the P content decreased from the apex towards the base of the fruit. In a similar study of internal breakdown, Burdon et al. (1991) observed that the P concentration was higher in disordered 'Kent' and 'Beverly' mangoes than in healthy fruit. Disordered tissue of affected immature fruit also had a higher P level than healthy portions of the mesocarp. Krishnamurthy (1981)

also found higher P concentrations in 'Alphonso' fruit affected by spongy tissue (0.19 g 100 g^{-1}) than in healthy fruit (0.12 g 100 g^{-1}). It is difficult to find an explanation for the presence of elevated P concentrations in disordered tissue. Phosphorus is a constituent of cellular compounds, notably nucleic acids and phospholipids (Bidwell, 1979). It plays an essential role in many biochemical processes including respiration and carbohydrate breakdown (Mengel and Kirkby, 1982). Internal breakdown is considered to be a ripening disorder (Subramanyam, 1971). As such, it involves a number of biochemical processes such as softening, climacteric respiration, and ethylene production (Gomez-Lim, 1997). Phosphorylases are among the enzymes involved in starch degradation by adding phosphate to glycosidic molecules forming monosaccharide phosphate (Smith, 1993). There is no detailed information on the production of these substances in disordered mangoes. However, it is possible that the high P concentrations observed in the disordered fruit tissues are related to increased demand for phosphate-based substances such as ATP in the ripening tissues due to an increased respiratory rate and/or accelerated degradation of starch molecules, making the disordered fruit stronger sinks for P than healthy fruit.

There were no differences in fruit K concentrations between disordered and healthy mangoes when the disorder first appeared or when the fruit were ripe. Young et al. (1962) and Malo and Campbell (1978) were also unable to correlate internal breakdown with leaf K levels. However, Burdon et al. (1991) reported that K concentrations in the disordered mesocarp of mature green 'Beverly' mangoes were significantly higher than those in the healthy mesocarp. Krishnamurthy (1981) reported lower concentrations of K in internal breakdown-affected fruit compared to healthy fruit. The differences between each of those studies and between those studies and the present study may be due to differences in the maturity stage at harvest. Immature and mature green fruit were used by Burdon et al. (1991) whereas Krishnamurthy (1981) used fruit that had ripened off the tree. In the present study, fruit were collected on a

weekly basis until on-tree ripening occurred. Also, differences in K fertilization may have interfered with the fruit K concentrations observed in each study. No information was given on the fertilization programs applied to the orchards where the fruit samples were collected by Burdon et al. (1991) and Krishnamurthy (1981).

The differences in Mg concentrations between disordered and healthy fruit were not significant during most of the fruit development period. This result is in agreement with results previously reported for 'Alphonso' fruit affected by internal breakdown, where no differences in Mg levels were found between disordered and healthy fruit (Krishnamurthy, 1981). Agarwala et al. (1962) reported higher Mg concentrations in the distal region of mango fruit affected by black tip compared to the intermediate or proximal portions. However, black tip may be a mango fruit disorder that is unrelated to internal breakdown. Burdon et al. (1991) observed that the disordered mesocarp of fruit with internal breakdown had higher Mg levels than healthy mesocarp. Further studies are needed to determine the role of fruit Mg concentrations in internal breakdown of mango fruit.

Concentrations of Zn, Mn, and Fe did not significantly differ between disordered and healthy fruit throughout the sampling period, whereas the B concentration in disordered fruit was significantly higher than that of healthy fruit at 8 WAFS. The higher Cu concentrations in ripe, healthy fruit compared to disordered fruit may be more related to fungicide application rates than to the incidence of the internal breakdown. Many of the fungicides used to control anthracnose of mango in south Florida contain Cu. Agarwala et al. (1962) did not find a significant relationship between the concentrations of Fe, Mn, or Cu and the incidence of the black tip disorder in 'Safeda' and 'Tamboori' mango fruit. The lack of differences in Zn, Mn, Fe, and B between disordered and healthy fruit suggests that these micronutrients do not play a significant role in the incidence of internal breakdown.

Conclusions

Indisputable evidence for the role of any nutrient element including Ca in the occurrence of internal breakdown is lacking. No relationship between fruit Ca concentrations and internal breakdown was found. Thus, this study does not support the hypothesis that internal breakdown results from Ca deficiency. Further studies are needed to elucidate the role of mineral nutrition in the development of internal breakdown of mango fruit. Such studies should include the use of radioactive markers for Ca and possibly other elements so that the incorporation of these elements in different tissues of the fruit could be followed. Fruit nutritional studies with mature mango trees in containers may be essential to determine the role of mineral nutrient elements in internal breakdown. In container studies, specific elements could be withheld from the tree to determine if deficiency of one or more elements results in the development of internal breakdown. Except for P and Cu, there were no significant differences in mineral element concentrations between disordered and healthy fruit when the fruit were ripe. At the early stages of internal breakdown, N, P, Ca, and B concentrations were significantly higher in disordered fruit than in healthy fruit. These results indicate that a nutritional imbalance established early during fruit ontogeny may be responsible for internal breakdown. It is also possible that an unknown factor triggered the biochemical processes that resulted in those elevated N and P concentrations in the disordered fruit, resulting in the early ripening of the mesocarp.

CHAPTER VII

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Throughout fruit ontogeny, significant changes take place in the nutritional status of mango leaves and fruit. Calcium and Mg concentrations in the leaves fluctuated more than the concentration of other macronutrients, whereas the concentrations of most nutrients in the fruit decrease throughout fruit ontogeny. There is an inverse relationship between leaf and fruit Ca which translates into an increasing leaf to fruit Ca ratio with time. The anatomical differences between leaves and fruit may predispose leaves to be stronger sinks for Ca than fruit. By reducing the transpiration rate of leaves with antitranspirants or by reducing the total leaf area with defoliants, it may be possible to divert some of the Ca present in the transpiration stream towards the fruit, which might result in increased fruit Ca concentrations.

Younger fruit contained higher concentrations of mineral elements than mature or ripe fruit. The decrease in the nutrient concentrations in the mango fruit observed during fruit development may be attributable to differences between the rate of nutrient uptake and the fruit growth rate. It is also possible that the decreasing nutrient concentrations over time are due to accelerated fruit growth and cell enlargement following fruit formation. The greatest declines in the fruit mineral element concentrations occurred between four and eight WAFS. That may be the critical period for the onset of nutritional deficiencies in the fruit and, consequently, the period at which the fruit have a higher need for mineral elements. Thus, applications of mineral elements beginning early in fruit development may help to prevent or minimize the

incidence of mineral deficiencies in mango fruit, especially those nutrients with low mobility such as Ca.

Foliar applications of $CaCl_2$, Cab'Y, or Packhard beginning 4 WAFS did not increase the Ca concentrations of mango fruit. However, there were significant relationships between Cab'Y and Packhard concentrations and the concentrations of several other nutrients in mango fruit, especially B. Foliar applications of $CaCl_2$, Cab'Y, or Packhard also did not reduce the percentages of disordered fruit. The presence of a thick, waxy cuticle on the fruit exocarp may have prevented Ca from penetrating into the fruit inner mesocarp, which is the area of the fruit most affected by internal breakdown. The penetration of Ca into the fruit may have improved if the trials had begun at earlier stages of the mango fruit ontogeny. During the early stages of fruit ontogeny, the cuticle is probably poorly developed, and a thinner cutin layer could allow better penetration of sprayed Ca into the fruit.

No definitive evidence on the role of Ca in the occurrence of internal breakdown was found in these studies. In the past, contradictory results have been reported from the use of $CaCl_2$ for controlling spongy tissue in 'Alphonso' mango. The application of $CaCl_2$ to control other physiological disorders in other plants species was not always successful. The relative efficiency of $CaCl_2$ may not be related to the chemical itself, but may be related to anatomical characteristics inherent to the plant species under study. Packhard and Cab'Y significantly increased fruit B concentrations throughout the fruit development period. These materials may represent reliable sources for overcoming B deficiency in affected plants.

The exact cause of internal breakdown in mango fruit remains to be elucidated and may remain controversial for some time. For the moment, it is not known if the disorder is due to a mineral deficiency or if environmental or genetic factors are involved. However, the presence of higher numbers of Ca crystals in disordered tissues

of fruit with stem-end cavity than in healthy tissues may constitute additional support for the hypothesis that Ca deficiency is the cause of internal breakdown of mango.

Fruit of 'Irwin' were less severely affected by internal breakdown than those of 'Tommy Atkins' or 'Van Dyke'. Information in the literature indicates that there is cultivar-related susceptibility or resistance to the disorder. There could be differences in the ability to utilize or concentrate specific minerals between susceptible and tolerant or resistant cultivars. Thus, it may be possible to minimize the disorder in susceptible cultivars by early applications of the deficient mineral. In addition, studies involving the use of radioactive markers would be useful in determining the exact path and partitioning of applied mineral elements in the plant tissues.

Differences between susceptible and resistant cultivars to internal breakdown may also be anatomical. There are differences in the amount of fruit fibers among mango cultivars. There could also be differences in the tensile strength of the fibers. Internal breakdown can be microscopically characterized by a degeneration of the vascular tissues in the interior mesocarp and in the proximal region of the fruit. The dissolution of the vascular tissues and the breakdown of the cell walls point to a possible involvement of degrading enzymes in the disorders. It is not known if these enzymes are activated by the degeneration process or if they are responsible for the dissolution of the vascular tissues.

Based on anatomical observations, jelly seed, soft nose, and stem-end cavity appear to be different disorders. However, it is possible that all three disorders are caused by the same factor. The first symptoms, particularly for jelly seed and stem-end cavity, are present early during fruit development. Therefore, control measures for preventing or reducing the incidence of these disorders in mango should target the early stages of fruit development.

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BIOGRAPHICAL SKETCH

Luc Raymond was born in Limbé, Haiti, on October 18, 1952 as the third of five children. He was raised in Cap-Haitien, Haiti, where he graduated from elementary school in 1965 and from secondary school in 1972. In 1976, he received a B.S. in Agriculture from Faculté d'Agronomie et de Médecine Vétérinaire (FAMV), Port-au-Prince. From 1976-1980, he worked on an agricultural development project (Bureau de Nutrition/USAID) in Haiti. He was awarded a scholarship by FAMV and Agence Canadienne pour le Développement International (ACDI) and received an M.S. degree in horticulture from the University of Puerto Rico, Mayaguez, in 1983. Following graduation, he taught Plant Propagation and Tropical Fruit Crops at FAMV from 1983-1993. In 1993, he began a Ph.D. program in horticulture at the University of Florida, with the sponsorship of FAMV, USIS (United States Information Service), and Latin American Scholarship Program for American Universities (LASPAU). He received his Ph.D. in horticultural science from the University of Florida in 1997. Dr. Raymond's professional interests focus mainly on teaching horticulture-related subjects, improving agricultural education at FAMV through introduction of graduate programs, and implementing policies for protecting the environment and improving the agricultural production of Haiti.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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